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EXPERIMENTAL MEDICINE

ON THE PIGMENT OF THE NEGRO'S SKIN AND HAIR.

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PLATE XIV.

1. Introduction, p. 361—2. Earlier work on the pigment of the negro's skin, p. 363—3. Methods of obtaining and cleansing the epidermis, p. 364—4. Action of reagents on the cleansed epidermis, p. 365—5. Methods of isolating the pigmentary granules, p. 367—6. Possible contamination of the pigmentary granules with bacteria and cell fragments, p. 370—7. Proportionate amount of pigmentary granules in the dried epidermis, p. 371—8. The substratum of the pigmentary granules p. 372—9. The inorganic constituents of the granules, p. 376—10. Methods of isolating and purifying the pigment, p. 381—11. The pigmentary granules and the pigment of the negro's hair, p. 383—12. Alkali-albuminates and peptone-like bodies formed by the action of alkalies on the epidermis and hair, p. 384—13. Chemical properties of the isolated pigments, p. 386—14. Combustion analyses, p. 389—15. The iron content of the isolated pigment, p. 393—16. The origin of the pigmentary substance, p. 395—17. Total amount of pigment and of pigmentary granules in the skin of one negro, p. 396—18. Function of the epidermal pigment, p. 397—19. Conclusions, p. 399.

1. INTRODUCTION.

THE pigments of the animal body constitute a numerous and widely distributed class of substances. Almost all of the tissues and fluids contain more or less pigment either in solution or, what is more frequently the case, in the form of minute granules of various shapes.

Some of the pigments that are of great physiological importance—hæmoglobin and the biliary pigments, for example—have been the subject of careful investigation and are fairly well understood with respect to their physical and chemical properties, their origin and relationships, and the physiological functions which they subserve. There are many more pigments of the human body whose chemical properties are somewhat known, but whose physiological relationships are very obscure. As belonging to this class may be named the pigments found in melanotic tumours, those of the retinal and choroid coats of the eye and of the hair, and the colouring matters of serum, of the urine and of fats.

Another large class of coloured substances is still less understood. Of these substances we know at present little more than that they exist in this or that tissue of the body; not one of them has been the subject of extended chemical research. Instances of these are the dark pigments * normally present in the heart, liver, kidney, thyroid gland, suprarenal body and testicles, on the peritoneal surface of the intestines, in the pia mater and in nerve cells.

Prominent among these little-known pigments is that found in the epidermis of the dark-skinned races. This pigment is of interest not alone because it is the distinguishing characteristic of the great majority of the human race and because for them it serves a physiological purpose, but also because of its very probable relationship to the pigment more sparingly deposited in the skin of the so-called white races and to the dark pigments known as melanins, examples of which are found in the hair and in certain malignant tumours.

Should we reach a thorough understanding of the pigment of the negro's skin, and should this pigment prove to be identical with that in the skin of the white races, this knowledge might throw great light on many anomalous cases of pigmentation; for example, the bronzed skin of Addison's disease, the brownish patches appearing on the face and other parts of the body during pregnancy, the brown or black patches sometimes covering large areas of the body and known as

* Compare Maass, Zur Kenntniss d. körnigen Pigmentes im menschl. Körper. *Arch. f. mikr. Anat.*, Bd. xxxiv, p. 452.

nævi spili, nævi verrucosi, and nævi lenticulares, and the lesser pigmentations called freckles.

Perhaps also closely allied in chemical composition to these pigments is that which is developed in the course of various chronic skin diseases, such as psoriasis, lichen ruber, and xeroderma pigmentosum; also that due to chemical irritation, as after the application of mustard or cantharidal plasters or that which follows the repeated inroads of clothes lice and other parasites.

We shall show in the following paper that it is possible to isolate the pigment of the negro's skin, and we believe that the same or a similar method will be found applicable to many of the instances of pigmentation above enumerated, more particularly to extensive discolorations of the skin in white persons, as in Addison's disease.

2. Earlier Work on the Pigment of the Negro's Skin.

The only work of a chemical nature known to us on the pigment of the negro's skin is found in a brief but suggestive paper communicated in 1876 to the Chemical News by Prof. J. W. Mallet,* on behalf of his pupil Dr. F. P. Floyd. This investigator confined himself to a study of the pigment in its crude state—that is, the pigmentary granules plus the cell structure enveloping them. His material was obtained by cleansing with water scrapings of the skin and treating them with alcohol and ether to remove the fatty matters. Since no attempt was made to isolate the true colouring matter, or even the pigmentary granules themselves, the results arrived at are of very restricted value. Two instances will suffice to show how inapplicable to the isolated pigment is a statement which may be perfectly true of the pigment while still contained in the cells of the epidermis.† Floyd says that the "colouring matter" is insoluble in dilute alkalies, but we found the purified pigment to be soluble in the most dilute alkalies or alkaline carbonates or even in distilled water, provided only that the pigment used has been recently precipitated.

* On the Chemical Character of the Pigment of the Negro's Skin. *Chemical News*, 1876, vol. xxxiv, p. 179.

† The term pigment-granules in Floyd's paper can only be construed to mean the pigment-bearing cells of the rete mucosum.

Again, the main point in Floyd's paper and the one for which he has been widely quoted is his conclusion regarding the iron content of the pigments. This point, however, can not be settled by an analysis of strips of cuticle. On incinerating strips of dried cuticle taken from both black and white cadavers, he found that the ash from the former weighed approximately twice as much as that obtained from an equal quantity of the latter, and that it contained approximately twice as much iron. From this he concluded that in all probability the pigment of the negro's skin is a chemical modification of the red colouring matter of the blood. But it must be remembered that ferruginous, colourless compounds of a proteid character are present in every cell, and on that account no conclusion as to the iron contents of the pure pigmentary substance can be reached by Floyd's method. We shall show later that in the process of purifying the pigment, the amount of iron steadily decreases with the removal of the adherent inorganic compounds till only the slightest traces of this element are left—so small an amount, indeed, that we have concluded that the pure pigment contains no iron as a constituent of its molecule.

3. Method of obtaining and cleansing the Epidermis.

The method of obtaining the cuticle in which the pigment is lodged varies according to the state of the cadaver. When it has lain for some time in contact with aqueous fluids, the epidermis may easily be peeled off in large patches from the underlying corium and, what is a further advantage, many of the hairs remain embedded in the corium. Or the entire skin is first removed and then cut into pieces of manageable size and scrubbed with a stiff brush under a forcible jet of water, after which each piece is held for a few minutes under boiling water and then immediately subjected to a stream of cold water, a process which it may be necessary to repeat once or twice. The consequent expansion and shrivelling results in a separation between the rete mucosum and the underlying corium, and when the pieces are stretched out and fastened on a board the entire epidermis can be removed with ease, much as a piece of wet paper

is lifted from a table on which it has been flattened out. The corium, it may be added, appears perfectly white after the epidermis has been removed.

When these methods of peeling off the epidermis are not possible, the following method may be used. Large pieces of skin obtained from the dissecting room are allowed to soak for three or four days in a five- to seven-per-cent solution of potassium hydrate, after which the epidermis is removed by scraping with a nickel spatula. This method is inferior to those before mentioned on account of the greater difficulty in removing the hairs.

When a sufficient quantity of epidermis has been collected, it is treated first with water, then with alcohol and ether in large extractors especially constructed for the purpose. After ten days to two weeks of this treatment the shrivelled and dried epidermis has become grayish white on its upper surface, the whole appearance reminding one of paper made by wasps, and on its lower side black or brown according to the amount of pigment contained in it. The hairs are now carefully removed, a tedious process requiring many days of labour.

When no more hairs can be detected, the microscope reveals that the small white specks with which the lower surface is studded consist of dried hair follicles in each of which is embedded a minute hair. These hair follicles may also be removed with the help of broad nickel forceps, but this process requires so much time that it was only used in preparing the epidermis for the quantitative estimation of the pigmentary granules.

4. Action of Reagents on the Cleansed Epidermis.

The epidermis cleansed as described was subjected to the action of various chemical reagents as follows:

Alkalies.—Dilute solutions of the free alkalies (five to fifteen per cent) allowed to act on the epidermis at room temperature for some days will disintegrate it, and maceration at water-bath temperature brings about a very complete disintegration in a few hours. This is, however, no true solution of the pigment, for although a

large part of the turbid fluid will pass through filter paper with large pores, it clears up, if allowed to stand for a day or two, a closely packed precipitate being found at the bottom, while the fluid above is of a faint straw-colour or ruby-red, according to the strength of the alkali used (five to ten per cent) and the length of time it has been applied (two to six hours). This black sediment falls out more quickly if the fats have been thoroughly removed from the epidermis before maceration.

Even prolonged boiling with ten- to twenty-five-per-cent solutions of the alkalies extracts but very little of the pigment. Whatever the strength of the alkaline solutions used, the supernatant fluid, on being filtered and neutralized with an acid, throws down a precipitate that is found to contain a great deal of proteid matter which is presumably an alkali-albuminate with the admixture of a little pigment.

If the black precipitate that settles out from the turbid fluid as above described is collected on a flat filter and washed with a very weak solution of acetic acid, it will be found to consist of minute black or brownish granules which resemble the pigmentary granules seen in the lower cells of the cuticle in a microscopic section of the skin. Although the granules are thus resistant toward even strong solutions of the free alkalies, they dissolve readily in weak solutions of the alkalies under a steam pressure of 150° to 160° C. Thus it was found that when scraps of cleansed epidermis are heated in a sealed tube with a five-per-cent solution of potassium hydrate for a few hours at the above temperature, a clear black fluid is obtained which passes through filter paper with great ease and can be diluted with water to any extent without causing a precipitate. The addition of an acid to this solution throws down a black or brown flocculent precipitate which is found to possess the properties of a weak acid; it forms very soluble amorphous salts with free alkalies or their carbonates.

Acids.—With the exception of nitric acid, dilute solutions (five to fifteen per cent) of the mineral acids allowed to act at room temperature do not dissolve out the pigment. Concentrated sulphuric

acid requires weeks to disintegrate the dried epidermis. For the first ten days the only effect is to swell the pieces of epidermis and bring out all their grooves and ridges. Even after two months thin filmy pieces are seen swimming in a dark fluid out of which settles no precipitate.

Concentrated hydrochloric acid is more effective; under its action in the course of two weeks all of the pigmentary granules have settled to the bottom, leaving the supernatant fluid but slightly coloured.

Concentrated nitric acid and fuming nitric acid are most effective solvents of the entire epidermis, the pigmentary granules included. After some hours a dark-brown fluid results which, on being freely diluted with water, throws down a dark-coloured flocculent precipitate. This precipitate, washed with alcohol and ether, is found to dissolve with ease in very dilute alkalies, yielding an intensely black solution. But if the nitric-acid solution has been allowed to stand for some weeks, dilution with water no longer throws down the precipitate and the solution has in the meantime become almost decolourized. Boiling the epidermis for a short time with much nitric acid of specific gravity 1.19 yields a dark-brown solution which, on the further application of heat, turns yellow and becomes finally almost entirely colourless. On diluting this solution with water, no precipitate is thrown down.

Glacial acetic acid allowed to act for months produces no marked changes to the naked eye; under the microscope, however, it becomes evident that certain changes in the cells have taken place, for the pigmentary granules are seen with greater distinctness. The action of chlorine and other oxidizing agents on the epidermis is the same as their action on the isolated pigment, which will be described later.

5. Methods of isolating the Pigmentary Granules.

From the experiments described in the foregoing pages it will be seen that the pigmentary granules of the epidermis must be classed with the most resistant and unalterable elements of the body.

For the purpose of learning what substances enter into their composition, we proceeded to isolate them by the following methods: A given weight of the epidermis, varying from 10 to 160 grammes, cleansed as before described, even the dried hair follicles being for the most part removed, was submerged in not less than twenty times its weight of a five-per-cent solution of pure potassium hydrate contained in large nickel bowls placed on the water bath.

The water in the bath was kept actively boiling, and the pieces of epidermis were for the first half hour constantly stirred with a nickel spoon; under these conditions the disintegration of the epidermis proceeded at a rapid rate, at the end of half an hour only very small particles remaining undissolved.

From time to time water was added so that the concentration of the solution should not go above five or six per cent. If after this process has been carried on for six hours the nickel bowl be set aside for twenty-four hours, there will be found in it a supernatant fluid of light-reddish-brown colour and a dense, black powdery precipitate consisting of the pigmentary granules.

Microscopical examinations were frequently made in order to follow the gradual disintegration of the epidermis step by step until there remained only minute brownish particles, not to be clearly distinguished except with the help of an immersion lens.

In Plate XIV, Fig. 1, we have given a drawing of a single cell detached from its neighbours showing the appearance of the granules. Fig. 2 represents the state of the disintegration after the potassium hydrate has acted on the epidermis for about two hours and a half. It will be seen that all cell outlines have been destroyed, the granules adhere together, forming brown or black lumps in each of which the individual granules are easily detected, and now and then a small aggregation of granules has a contour suggestive of the original cell form. Fig. 3 represents the condition after six hours of maceration. Light pressure on the cover glass causes the granules to fall apart and to appear in the field as discrete but very minute light-brown particles. As to the shape of these granules, we have concluded that they are rather elongated than round; this, however, can only

be determined by examining them under an enlargement of a thousand diameters or more and under favourable circumstances. The traces of foreign matter that may still be detected among the granules after long maceration may be removed by washing with warm hydrochloric acid, alcohol, chloroform, and ether. Thus treated, dried, and powdered, the granules cohere into little black lumps, as shown in Fig. 4. Rubbing up some of this powder with dilute alkalies gives us the picture under the microscope of individual granules, as seen in Fig. 3.

A few yellowish ovoidal and globular particles seen here and there were at first assumed to consist of a substance related to keratohyalin, but their inability to take up methylene blue and their disappearance in hot ether and hot alcohol show that they consist of fat. Small irregular black lumps and long black rods that were now and then found among the granules were shown by check experiments with pure keratin to consist of nickel sulphide. This is formed by the sulphur which is split off from the keratin acting on the nickel of the bowl and forming a thin coating of nickel sulphide, which breaks up into singular and varied forms.

Another and very simple method of isolating the pigmentary granules consists in the use of concentrated hydrochloric acid, which, as already described, entirely dissolves the epidermis excepting only the granules, which fall to the bottom as a black sediment. Treatment with so powerful an agent may remove from the granules much of their inorganic matter, but when it is desired to isolate the pigment itself rather than the pigmentary granules, this method promises to be of service because it enables us to isolate with ease any desired quantity of the granules, no matter how large.

The black granules, prepared by either of the above methods, washed and dried, become a snuff-coloured powder, not to be distinguished in appearance from that yielded by the pigment itself when it has been isolated from the granules.

But, as we shall see later, these two powders differ markedly in their behaviour toward reagents.

6. Possible Contamination of the Pigmentary Granules with Bacteria and Cell Fragments.

It might be supposed that the black precipitate described by us as consisting of the ultimate pigmentary granules would be found to be much contaminated with bacteria. Such is not the case, however. The usual methods of staining have failed to indicate their presence in sections of the dried and cleansed epidermis, and the following experiments were made to determine whether bacteria can retain their structural integrity when macerated in a five- to six-per-cent alkaline solution at water-bath temperature. In the first experiment 5 grammes of dried tubercle bacilli (pure culture) were suspended in 75 cubic centimetres of a five-per-cent solution of potassium hydrate contained in a small flask which was attached to a reflux condenser, and the mixture was then heated for four hours on an actively-boiling water bath. At the expiration of this time no bacteria could be detected either on direct examination of the resulting turbid fluid or by staining some of the precipitate thrown down by alcohol.

Nothing remained but amorphous flakes and stringy masses. A like negative result was obtained when scrapings from culture tubes containing the staphylococcus pyogenes aureus were subjected to the treatment just described. Nencki and Schaffer * have also shown that certain putrefactive bacteria (names not given) are largely dissolved when treated on the water bath with only 0.5-per-cent potassium hydrate, an insoluble, structureless residue remaining. Contamination of the pigmentary granules with bacteria must therefore be entirely excluded.

Neither can there be an admixture of cell fragments, for in the granules that have been collected on flat filters, washed with hot two-per-cent potassium hydrate, water, hot five-per-cent hydrochloric acid, hot alcohol, chloroform, and ether, the microscope fails to detect any but the merest traces of matter, excepting only the nickel sulphide obtained from the bowls. This statement is also confirmed

* Review in *Jahresb. d. Thier-Chemie*, Bd. ix, p. 385.

by the fact that the iron reaction (HCl and K_4FeCy_6) can now no longer be obtained.

But the most conclusive proof that no foreign matter of organic nature lies between the granules is found in the fact that from a given weight of granules an amount of crude pigment may be obtained by the method soon to be described whose weight equals that of the granules minus their ash. In other words, the crude soluble pigment with properties so different from the granules contains everything that they contain, barring only their great excess of ash.

The force of the above statement will become more apparent when we note the method of disrupting the granules, presently to be described, for the reagents used for this purpose are the very ones that have already done their work in dissolving out foreign organic matter.

7. Proportionate Amount of Pigmentary Granules in the Dried Epidermis.

Ten grammes of cleansed epidermis from which even the hair follicles had been removed were macerated for six hours in a nickel bowl as just described.

The pigmentary granules were then collected in two portions on small flat filters, washed with a warm, dilute solution of potassium hydrate, then with alcohol containing a very little acetic acid until the potassium hydrate, as shown by the flame test, was entirely removed, then with hot alcohol, hot chloroform and ether, and dried at 110° C. to constancy of weight.

The amount of pigmentary granules on the one filter weighed 0.2120 gramme, on the other 0.1089 gramme. The total residue of pigmentary granules, therefore, equaled 0.3209 gramme. From this amount we must subtract 0.0121 gramme as consisting of sulphide of nickel, leaving 0.3088 gramme of pigmentary granules. The granules would, therefore, constitute 3.08 per cent of the epidermis when cleansed as described.

In a second experiment 9.77 grammes of carefully prepared epidermis from various parts of the body was treated in the same man-

ner, except that the process of maceration was carried on in a porcelain instead of a nickel bowl. In this case the pigmentary granules obtained weighed 0.3693 gramme.

Neglecting the trifling weight of contaminating material that was derived from the porcelain bowl, we find the proportion of granules somewhat higher—viz., 3.78 per cent. It is well known that the epidermis varies in thickness in different parts of the body, and the difference in the above results is therefore easily accounted for.

8. The Substratum of the Pigmentary Granules.

We believe that a perusal of the following pages will leave no doubt that the pigmentary granules which settle in the bowls when the epidermis is macerated in warm solutions of potassium hydrate are no other than the pigmentary granules that may be seen under the microscope in every cross-section of the negro's skin. We grant that we must remain in ignorance as to whether the long maceration necessary to free the granules from the cells in which they are lodged may not have extracted from the granules themselves some constituent of a proteid or keratinoid character. It would appear probable, however, that had this been the case, the granules would have fallen to pieces as do bacteria when their soluble proteids are removed from them by treatment with hot alkalies.

We must also concede it to be possible that the water, alcohol, and ether used have removed from the granules some constituent soluble in these agents, but this error, if it be such, is unavoidable.

We have already alluded to the fact that the granules become somewhat translucent and pale after long treatment with hot alkalies. This change becomes very apparent when microscopic sections of healthy skin taken from a living subject are thus treated on the warm stage and examined from time to time. This has been kindly done for us by Dr. T. C. Gilchrist. Dr. Gilchrist has watched under the microscope the sections during the different stages of their disintegration under the action of alkalies of varying strength, and has demonstrated that the granules remaining are those originally deposited in the epidermis.

Post * has also made this observation, for he says that the elementary granules are seen as pale, somewhat swollen rods when they have been decolourized † by concentrated potassium hydrate which has been allowed to act on them at room temperature for some weeks.

Are we to conclude from these observations that the pigmentary substance is lodged in a substratum of a different character, that there is a ground substance which constitutes, so to speak, the body of the granules and which is permeated with the pigment? In other words, are we to look upon these granules as a kind of trophoplast, like chlorophyll granules, and like them composed of a substratum of proteid matter which is stained with a pigment?

It appears that histologists who have given attention to the subject incline to this latter view. W. v. Nathusius, ‡ for instance, has recently shown in his paper on the fibrillary structure of the keratinoid cells of the fibrous coat of hairs that these cells may be so far disintegrated with warm ammonia as to leave undissolved their pigmentary granules, lying for the most part in rows as originally deposited in the cells, but deprived of some of their pigment. This investigator also furnishes drawings of the granules arranged in rows in the isolated fibrils, their natural pigment removed, and methyl violet or fuchsin substituted.

Still other evidence that there is present in these granules a non-pigmented substratum is presented by Reinke, who has recently sought to establish the histological similarity of these structures to the trophoplasts of the botanist. Reinke has found that the pigmentary granules of the peritoneal cells of the larva of the salamander may be bleached with hydrogen peroxide and then stained with safranin. He concludes as follows: "Inasmuch as the pigmentary granules can

* Virchow's *Archiv*, Bd. cxxxv, p. 493.

† Post evidently does not use "Entfärbung" in its literal sense. In our experience, potassium hydrate never entirely removes the pigment unless the granules have first been subjected to an acid.

‡ *Arch. f. mikr. Anat.*, Bd. xliii, p. 153. In the explanatory remarks devoted to the drawings v. Nathusius refers to detritus lying among the fibrils. We must therefore assume that the ammonia leaves much undissolved matter in addition to the fibrils and granules.

* *Arch. f. mikr. Anat.*, Bd. xliii, p. 393.

be freed of pigment and again coloured with a staining dye, it would appear to be a justifiable conclusion that the substratum of the granules is something different from the pigmentary substance."

We must say, however, that Reinke's argument does not seem to us conclusive. The substance constituting the pigment may have been left *in situ* after having been bleached by the hydrogen peroxide and the substance that took up the new dye may be no other than the original pigmentary substance itself. In a word, Reinke has not proved that the hydrogen peroxide removes anything from the granules.

No objection can be made, we think, to the following method of demonstrating that there is in these granules a substratum of a non-pigmentary character. Some of the granules isolated and purified as already described are macerated for a week or ten days at room temperature with a large excess of hydrochloric acid of five per cent. The flask containing the granules and the acid is shaken a few times a day, and every second day the acid is poured off and fresh acid added. At the end of the time the granules are washed free of acid with the help of a suction pump or a centrifugal machine, and are then treated on the water bath with dilute (two- to five-per-cent) potassium hydrate. An astonishing change has taken place; the granules now yield up their pigment in large amount to weak alkalies and a fluid of a deep-brown, almost black, colour may be filtered off in a short time.

When examined under the microscope, immediately after the coloured substance begins to leave them, the granules may still be detected as mere shades of their former selves.

Soon, however, only faintly coloured *débris* is left, as when bacteria are similarly treated. This *débris* must originate in the pigmentary granules, and it can represent nothing else than the substratum of Reinke and others.

We have attempted to stain the granules just as they were beginning to give up their black pigment to the potassium hydrate. This would have made the proof of a separate substratum absolutely complete, for we should have had on the one hand the clear, black

solution, and on the other the minute elements coloured with the artificial dye. But after being treated with potassium hydrate, the ground substance seems incapable of taking up the new dye. We tried, therefore, another method. After the treatment with cold hydrochloric acid of five per cent, a small quantity of the granules was placed in a glass tube with a mixture of one volume of alcohol and six volumes of concentrated ammonia. The tube was sealed and then kept in a water bath at 100° C. for six to ten hours. This process extracts from the granules a little of their pigment and they will then take up gentian violet with avidity, as seen in Plate XIV, Fig. 5.

The certain presence of a non-pigmented ground substance in the pigmentary granules has led us to inquire whether it would not be possible to isolate from white epidermis and white hair minute elements which would correspond in every particular, except colour, to the black granules of the negro's skin and hair. We therefore subjected to the treatment already described such structures as white horn, white hair of the dog and of the rabbit, merino wool, and scales of epidermis from a person afflicted with ichthyosis. We found that white horn dissolved in five- to ten-per-cent solutions of warm potassium hydrate to a perfectly clear fluid. The other keratinoid structures yielded varying small amounts of a highly resistant proteid. We have boiled this substance for four hours with a five-per-cent solution of hydrochloric acid with the result that only a part of it went into solution, and if the substance be again subjected to hot potassium hydrate of five per cent, only a little passes into solution. In short, even the alternate application for a long time of boiling alkali and acid does not entirely dissolve this resistant proteid.

We assume this body to have properties very like that of the ground substance of the granules, and if this assumption be correct, it explains why it is so difficult to remove from the pigment all traces of the contaminating ground substance. Of all the keratinoid structures just referred to, white hair from the dog is the most resistant to alkalies and appears to contain the largest proportion of the unknown proteid.

As the quantity of this proteid is in any case small, a large amount

of material is necessary, and most of our experiments were made on merino wool.

We shall soon publish the results of our observations on the chemical properties of this hitherto overlooked proteid. We may say that by our present method of macerating merino wool and the other keratinoid structures with potassium hydrate this resistant proteid is left behind, not as white granules, but as an amorphous compound. After isolating the proteid just referred to, we found that v. Nathusius* has shown that when white hair is treated with a boiling aqueous solution of methylgreen, minute, deeply coloured particles may be detected which correspond in size and in position to the "air spaces" of Kölliker in white hair and to the pigmentary granules in coloured hair. Von Nathusius therefore concludes that the same histological element is present in both white and black hair, differing in the latter case only in its pigment.

9. The Inorganic Constituents of the Pigmentary Granules.

Having seen that the pigmentary units may be isolated in any desired quantity, it becomes of interest to learn what inorganic constituents enter into their composition. This problem is, furthermore, one of physiological importance, as bearing on the broad question of the distribution and localization of the inorganic constituents of the body.

Numerous difficulties have been encountered in the analysis of the granules obtained from the epidermis. Their ash content is found to be too high, when they are not washed with hydrochloric acid, to remove the amorphous material which appears to be precipitated among them by the alkali used. This material promptly gives an iron reaction with HCl and K_4FeCy_6—a test which was applied in vain to the granules. This foreign material may readily be removed by washing the granules with hot hydrochloric acid of two to five per cent; but this process deprives the granules themselves of some of the inorganic matter properly belonging to them. We have incinerated the granules both before and after washing with hot hydro-

* *Loc. cit.*, p. 152.

chloric acid, and find that in either case an error is introduced into the analysis, the total ash being too large in the first and too small in the second. The true value lies somewhere between the two. In the case of the hair granules soon to be described, the ash content obtained by incineration without previous washing with hydrochloric acid gives, in our opinion, a correct value, because no foreign matter appears to be precipitated upon them.

Stating for the present the results of our analyses in round numbers, we find that the inorganic constituents of the pigmentary granules of the hair dried at 110° C. without previous washing with hydrochloric acid make up rather more than eleven per cent of their weight. Washed with hydrochloric acid of five per cent, their ash content still amounts to nearly seven per cent.

The pigmentary granules of the epidermis dried at 110° C. without previous washing with hydrochloric acid show an ash content of twenty-six per cent. After thorough washing with hot hydrochloric acid this percentage is reduced by one half.

The great difficulty of entirely removing all adherent foreign matter from the epidermis of a cadaver causes us to view with suspicion this very high ash content of the epidermal granules, and we can not at the present writing assert positively, as we can for the ash content of the hair, that no foreign inorganic matter was present. But, as has been described, the greatest pains was taken to cleanse the skin thoroughly, and the results we give are taken from several accordant analyses.

The acids present in the granules are silicic, sulphuric, and phosphoric; the bases * are calcium, magnesium, and iron, the order of statement representing the relative amounts. Treatment of the granules with hot hydrochloric acid of five per cent reduces the salts of sulphuric and phosphoric acids to a low figure, only about ten per cent of these constituents remaining, while the percentage of silicic acid is but little affected. Iron constitutes over four per cent of the

* A little aluminium oxide was also found in the ash from both kinds of granules. We must, however, reserve for a later paper the question whether a small amount of aluminium is contained in the human epidermis and hair.

ash of the epidermal granules when these have not been washed with hydrochloric acid, or about one per cent of the dry granules.

A trace of this element is retained by the granules with great tenacity in spite of repeated treatment with hydrochloric acid; but, as we shall see later, iron does not constitute an integral part of the molecule of the pigment.

That silicon is present in the pigmentary granules of both the skin and hair, we can have no doubt. As found by us in the granules of the hair, for example, no foreign source can be claimed for it when we state that the hair was cut for us from the heads of clean negroes in the hospital wards; that it was most carefully picked over and washed with hot water, alcohol, and ether; that it was macerated in nickel bowls, and the granules collected on flat filters placed on perforated porcelain plates in glass funnels, the sides of the filters projecting upward along the wall of the glass funnels, so that the granules themselves were hardly in contact with the glass, and the silicates taken from the glass by the alkaline fluid would all be carried into the filtrate. Besides, the amount of silicon found is too large to be derived from the glass funnel, even if no care had been taken. A conclusive proof that silicates are present in the granules of both the epidermis and the hair, and that they pass into solution along with the pigment and adhere to it with great tenacity, is seen in the following fact. It is possible, as we shall see, to prepare from the epidermis a specimen of soluble pigment that shall contain no less than twelve per cent of ash, including the silicates. Such a pigment will, nevertheless, remain in solution in alcohol of eighty to ninety per cent for an indefinite period, provided only that a freshly precipitated pigment be used. But silicates that have been dissolved out of utensils of glass and porcelain by alkalies, when added even in small amount to an alcoholic solution of the pigment of the strength referred to, fall out promptly, as we found by actual trial, and carry down with them considerable pigment.

The above furnishes one of several proofs of the close union that exists between the substratum of the pigment and the inorganic constituents of the pigmentary granules. Other instances of this condition

are seen in the firm union that exists between the proteids of the body and the mineral compounds accompanying them.* In the vegetable world we find the same close connection existing between cellulose and mineral compounds, like silicates of iron and calcium. Small quantities of inorganic material still adhere to cellulose, even after repeated solution in ammoniacal cupric oxide and repeated precipitations and washings with hydrochloric acid, thus furnishing a striking reminder of the readiness with which our pigment remains in solution in alcohol, notwithstanding the considerable quantity of adherent mineral matter. Lange † concludes, as the result of his experiments in this field, that the mineral constituents which can not be entirely removed from cellulose are distributed in the membranes of the cell as particles of an insoluble compound of the element in question; and when the membranes are dissolved, these mineral compounds are held in suspension in the solution, and filtering holds back only a portion. Some such hypothesis may also explain the "close union" referred to above as existing among the constituents of the pigmentary granules.

That silicon should be found in the pigmentary granules of both the epidermis and the hair need not occasion surprise. Silicon has long been known to be one of the constituents of hair; it has also been found in the epidermal scales from a person afflicted with ichthyosis,‡ and traces of it exist in the urine. It is found to a considerable extent in the ash of feathers, and Henneberg # has found it in the blood of chickens to the amount of 0.0139 per cent. It is very probable that silicon may be proved to be an indispensable constituent of the integumentary structures, more especially of their pigmentary granules.

As to the function of these inorganic constituents, we can only offer surmises. It may be that they serve the purpose of a mordant, enabling the substratum of the granule to hold and "fix" the solu-

* Cf. M. Nencki, Bemerk. üb. d. sogenannte Asche d. Eiweisskörper. *Arch. f. exp. Path. u. Pharmakol.*, Bd. xxxiv, p. 334.

† *Ber. d. deutsch. chem. Gesellsch.*, Bd. xi (1878), p. 826.

‡ Halliburton, Chemical Physiology and Pathology, p. 566.

\# *Ann. d. Chem. u. Pharm.*, Bd. lxi (1847), S. 257.

ble pigment.* Closer study of this interesting question may perhaps furnish an insight into nature's method of dyeing. We have already said that it is only possible to dissolve the granules in weak alkalies at water-bath temperature when they have first been subjected to maceration with hydrochloric acid. Now, it can be easily shown that the acid removes only inorganic constituents from the isolated granules, and it would therefore seem that the presence of this inorganic matter is in some way essential to the retention of the pigment, since its removal renders the granules soluble in a medium which before failed to dissolve them.

The ash content of the granules remains high, even if large deductions are made, as in the case of those derived from the epidermis.

Our analyses are verified in this respect by one made many years ago by Scherer,† who includes in his analyses of proteids that of the pigment called by him the pigmentum nigrum oculi. To obtain it, the black pigment-bearing cells of the choroid coat of oxen's eyes were brushed off with camel's-hair brushes under water, allowed to settle, and were then strained through a filter of linen cloth, in order to remove any fragments of tissue; the inky-black filtered fluid was then evaporated, and the residue extracted with boiling alcohol and ether, and dried; 0.345 gramme of the dried substance gave 0.035 gramme of ash, or 10.1 per cent.‡ In Scherer's analysis the entire pigment-bearing cells of the choroid coat were incinerated; but had he isolated and analyzed the granules themselves, we have no doubt the ash content would have approximated that found by us in the hair. It seems highly probable that all pigmentary granules contain a great deal of inorganic matter.

* Such a theory must, however, take account of the fact that white feathers contain fully as much silicon as do those that are coloured, also that the feathers of birds living on grains contain more silicon than those living mainly on soft fruits. *Vide* Gorup Besanez, *Ann. d. Chem. u. Pharm.*, Bd. lxi (1847), p. 47.

† *Ann. d. Chem. u. Pharm.*, Bd. xl (1841), pp. 1-65.

‡ Scherer gives the ash as amounting to 9.8 per cent, but, according to his own figures, it is as above.

10. Methods of isolating and purifying the Pigment.

It is only necessary to apply chemical reagents in the proper sequence in order to dissolve out of the granules the pigment contained in them. Thus, if the granules that have settled out from the alkaline macerating fluid be washed free of alkali by decantation, and then digested at room temperature with five to ten per cent HCl for ten days, the acid being renewed every two days, and, after being washed free of acid, be now again subjected to the action of warm dilute alkalies, it will be seen that the alkaline solution becomes at once deeply coloured. After some hours of maceration on the water bath with five- to ten-per-cent solutions of potassium hydrate, only a small residue, consisting largely of inorganic matter, is left undissolved, and repeated digestion with ten-per-cent potassium hydrate reduces this undissolved residue to trifling proportions.

Instead of digesting the granules with hydrochloric acid at room temperature, the same result may be obtained by boiling them with the acid for four hours. This method was used by us only once for the special purpose of studying the effect on the iron content of the ash. At all other times only cold five-per-cent hydrochloric acid was used that we might run no risk of removing iron from the molecule of the pigment.

The black alkaline solution of the pigment is now diluted with an equal volume of water and filtered; it remains perfectly clear on standing, a minute sediment of finely divided sulphide of nickel being found at the bottom of the vessel. In our early work we precipitated the pigment out of its first alkaline solution by means of a large excess of a mixture of six volumes of alcohol and one of ether. After allowing the precipitate to settle for some days, the supernatant fluid was removed with the siphon, and the dark-brown precipitate was collected on flat filters, washed with warm alcohol, to which a little acetic acid had been added, redissolved with the help of a trace of potassium hydrate or of ammonia, and reprecipitated with alcohol and ether. This process was repeated seven or eight times. In the later stages ammonia was used to redissolve the pigment, and, after the solution had

been effected, the ammonia was driven away on the water bath before the solution was filtered. In such a case the pigment still remains in perfect solution after the removal of the ammonia. With the gradual removal of the potassium used to effect the first solution, it became, however, increasingly difficult to precipitate the pigment with alcohol. Finally, it remained in solution in a fluid containing not far from ninety per cent of alcohol, and, in order to precipitate the pigment, it was found necessary to add considerable acetic acid. This fact made it seem probable that the pigment would be found nearly pure, but incineration showed an ash content as high as 12.4 per cent. We were therefore obliged to abandon this method of isolating the pigment, and to reserve the precipitation with alcohol and ether for the later stages of the process, after the ash content had been reduced by alternate treatment with alkalies and acids. Hirschfeld,* toward the close of his valuable paper on the pigment of the choroid coat, regrets that he did not sooner observe that an excess of alcohol precipitates the pigment, as he might have used this method to isolate it.

Recently we have proceeded as follows: A large quantity of cleansed epidermis—say 100 grammes—is macerated in a number of large nickel bowls with twenty to thirty times its weight of a five-per-cent solution of potassium hydrate. After the maceration has continued for six hours, the black fluid is poured into a large glass jar, and six volumes of alcohol are added. In a day or two a dense black precipitate has settled out, while the keratoses and alkali-albuminates derived from the epidermis remain in solution. The black precipitate is next subjected to the action of cold dilute hydrochloric acid, as already described. The granules are then dissolved in potassium hydrate in nickel bowls on the water bath, the solution is diluted with water and filtered, and the pigment is precipitated out by the addition of acetic acid in excess. After the pigment has settled out, the red, supernatant fluid is decanted, and the pigment is collected on flat filters and washed free of acid. For this purpose water is at first used, but, when the pigment begins to dissolve in the wash water, absolute alcohol is substituted. The pigment is now again dissolved

* *Zeitschr. f. physiol. Chem.*, Bd. xiii, p. 420.

in dilute potassium hydrate (two to five per cent), heated in the water bath for some hours, and again filtered, when a small residue of insoluble flocks will be found on the filter; it is then again precipitated with an excess of acetic acid, allowed to stand as before, collected, and washed. It is now dissolved in ammonia, the ammonia driven off on the water bath, the solution filtered, and the pigment precipitated out with alcohol and ether, to which a very little acetic acid has been added. This process is repeated until it is found that the ash content has been reduced to one or two per cent. After this, the pigment is washed with hot alcohol, chloroform, and ether until these reagents remove nothing further from it. The ash content is thus reduced to a low figure, varying from 0.8 to 1.2 per cent in the instances where the pigment was purest.

11. The Pigmentary Granules and the Pigment of the Negro's Hair.

While the study of the pigment of the negro's skin has been the chief purpose of our investigation, we have also isolated the pigmentary granules and the soluble pigment of the negro's hair. From 600 to 1,000 grammes of negro's hair, cleansed with great care and treated with alcohol and ether in large extractors, were macerated in large copper kettles over a free flame with ten times their weight of a five-per-cent solution of potassium hydrate, care being taken not to allow the temperature to rise above 95° C., or the solution of potassium hydrate to go beyond a concentration of six per cent. The black, turbid fluid was then poured into glass jars containing a large excess of alcohol or a large quantity of dilute hydrochloric acid of such strength that, when the turbid fluid had been added, the whole should have a plainly acid reaction. If alcohol is used, the black precipitate is flocculent; if hydrochloric acid is used, the precipitate falls out in large lumps that have a sticky feel, because of an admixture with an alkali-albuminate of a glue-like character. In this latter mixture sulphuretted hydrogen is also given off in large quantities, which is not the case when the turbid alkaline fluid obtained from the skin is similarly treated. The main constituent of the precipitate in both

cases is the pigmentary unit of the hair, a large black, rod-like particle, very much resembling in form certain bacteria. Plate XIV, Fig. 6 shows these rods in a piece of crushed hair, which had been macerated in hot potassium hydrate for fifteen minutes—a length of time found sufficient to remove the scaly covering of the hair and soften its fibrous substance. Fig. 7 represents the pigmentary granules of the hair after treatment in a closed tube with alcohol and strong ammonia, and subsequent staining with gentian violet.

In order to isolate the pigment of these granules, the black precipitate thrown out by alcohol or hydrochloric acid is treated in the way already described as applicable to the pigment of the skin. The pigment finally obtained is not distinguishable in any way from that obtained from the epidermis. As with the epidermis, so also with the hair, maceration in concentrated hydrochloric acid in a closed bottle entirely dissolves all of it but the granules. Twenty cubic centimetres of hydrochloric acid thrown on a few curls of negro's hair entirely disintegrate them in the course of a week. At the end of this time the supernatant fluid has a reddish-violet colour, while a black precipitate, consisting of the typical granules, has settled at the bottom. On account of its convenience, we propose to use this method in future work on the pigment of the skin and hair.

To our great surprise, a given weight of hair contains a smaller proportion of pigmentary granules than does a like weight of epidermis. Thus, 10 grammes of negro's hair yielded only 0.1889 gramme of granules, or 1.9 per cent, as compared with 3.08 to 3.78 per cent of granules contained in the epidermis. This result was afterward abundantly verified in the isolation of pigment from large amounts of hair.

12. Alkali-albuminates and Peptone-like Bodies formed by the Action of Alkalies on the Epidermis and Hair.

When the alcoholic fluid which is decanted from the granules is freed of its alcohol by distillation, and the resulting alkaline fluid is made acid, an alkali-albuminate, already referred to as having a ropy, sticky character, is thrown out. When the macerating alkaline fluid

containing either hair or skin granules is put into weak hydrochloric acid, in order to precipitate the granules, this alkali-albuminate at first remains in solution, although the fluid has an acid reaction, and can be precipitated by the addition of more hydrochloric acid to the fluid filtered off from the granules. N. Sieber * has also called attention to this substance in her paper on the pigment of the choroid coat and of the hair. In the case of the epidermis, an alkali-albuminate is also formed, but its physical properties are entirely different. It falls out as a powdery sediment, and does not form a translucent glue-like mass on drying, as does the alkali-albuminate from the hair. Both of the alkali-albuminates, when collected on filters, washed, and dissolved in a little alkali, now fall out immediately on neutralization with a mineral acid, not requiring an excess of acid, as they do when still mixed with the alkali used for maceration and with the soluble products of the epidermis and hair. They also dissolve readily in a little hydrochloric acid of twenty-five per cent, and are completely precipitated from this solution by the addition of ammonium sulphate or sodium chloride.

We think it highly probable that these albuminates are derived, in large measure, from the keratins of the skin and hair by the action on them of the potassium hydrate; for Lindwall † has shown that when the keratin obtainable from the shell membrane of the hen's egg is treated with sodium hydrate, an alkali-albuminate and peptone-like bodies are formed. We may also add that the fluids from which the alkali-albuminates referred to have been separated, all promptly give the red biuret reaction at room temperature, and, as the fluid no longer contains a native proteid or an alkali- or acid-albuminate, it is safe to conclude that we have in solution some peptone- or albumose-like body in addition to the alkali-albuminate referred to.

* *Arch. f. exp. Path. u. Pharmakol.*, Bd. xx, p. 364.

† Beiträge zur Kenntniss des Keratins. Reviewed in *Jahresb. d. Thier-Chemie*, Bd. xi, p. 38.

13. Chemical Properties of the Isolated Pigments.

The isolated pigment of the negro's skin presents the appearance of a dark-brown or snuff-coloured powder. If a little of this powder is placed upon a strip of blue litmus paper and moistened with a drop of water, it gives a markedly acid reaction. If it be heated in a platinum crucible, a mixed odour of burnt horn and pyrrol is perceived and the pine-sliver reaction for pyrrol is obtained, with such promptness and intensity that we must assume this substance to be present in considerable quantity among the distillation products of the pigment. Toward the close of the incineration the odour of pyrrol disappears, and an overpowering odour of hydrocyanic acid is given off. Freshly precipitated and washed free of potassium, the powder is soluble in water and in a large excess of alcohol of ninety per cent. Allowed to stand over sulphuric acid for months, or dried to constancy of weight by heating at 120° C., the pigment changes from snuff-colour to a dense black, its particles shining like anthracite coal. It loses its free solubility in water, alcohol, and dilute alkalies, but long-continued digestion on the water bath with strong alkalies again dissolves it.

The pigment is entirely insoluble in the usual organic solvents, as ether, chloroform, amyl alcohol, acetone, glycerin, acetic ether, xylol, toluol, carbon disulphide, phenol, and pyridine. It is precipitated from its aqueous solutions by potassium ferrocyanide, copper sulphate, ammoniacal zinc chloride, silver nitrate, lead acetate, calcium acetate, barium hydrate, and calcium hydrate. The addition of sodium chloride, magnesium or ammonium sulphate, and of other neutral salts to an aqueous solution induces a complete precipitation of the pigment. Alkaline solutions examined with the spectroscope show no absorption bands.

The addition of an acid in excess to the alkaline solutions causes the pigment to fall out at once as a brownish-black flocculent precipitate. Concentrated hydrochloric acid does not dissolve the pigment, and appears to have no damaging effect on it, even long boiling with a twenty-five-per-cent solution leaving the pigment unchanged. Con-

centrated sulphuric acid only slowly dissolves a little of the pigment, and, when the solution of what little is taken up is dropped into ice water, the pigment falls out apparently unchanged.

Concentrated nitric acid easily dissolves the pigment, forming with it a dense black solution, from which it may be precipitated on dilution with water. It may be boiled with this acid or repeatedly evaporated with it without loss of colour. Glacial acetic acid does not dissolve the pigment.

Very dilute solutions of the pigment lose their colour when acted upon for a few days by neutralized hydrogen peroxide, and dark-brown flocks settle out. Chlorine passed into an alkaline solution of the pigment immediately deprives it of its colour. When the pigment is repeatedly evaporated down in a porcelain bowl with nitro-hydrochloric acid, there remains a reddish-yellow resinous mass, insoluble in alkalies, water, alcohol, or ether, which dissolves readily in amyl alcohol. On evaporation, a reddish-brown resinous mass is obtained, the nature of which we have thus far been unable to determine.

The behaviour of the pigment toward reagents, as described, makes it evident that it is a very resistant substance. This view is still further strengthened by the following experiments: 2.6 grammes of hair pigment, with an ash content of 2.19 per cent, and a sulphur content of 1.7 per cent, was dissolved in 500 cubic centimetres of a five-per-cent solution of potassium hydrate, and kept for four and a half hours in an autoclave at a temperature of 185° C. At the end of this time the black solution was filtered, the pigment thrown out with acetic acid, collected, washed, and dried. On examination, it was still found to contain much sulphur. It was therefore again dissolved in 500 cubic centimetres of a five-per-cent solution of potassium hydrate, and kept for three hours at a temperature of 200° C. The solution not having changed in colour, it was brought up to a potassium hydrate content of thirty per cent, and was again kept for two hours at a temperature of 200° C. The black solution was then treated in the usual way to recover the pigment. As nearly as we could judge, fully one third of the pigment originally put into the

autoclave had disappeared, and the part left had more markedly acid properties than before. The recovered pigment was found to have a somewhat higher sulphur content. Thus, 0.3551 gramme gave 0.0496 gramme of barium sulphate, and therefore contained 1.9 per cent. of sulphur. Unfortunately, part of the solution from which the sulphuric acid was to be precipitated was spilled, and there has been no opportunity to repeat the experiment, so that we must place the sulphur content somewhat higher. We judge it to be in the neighbourhood of 2 per cent. We conclude that the proteid material of the ground substance had been decomposed by this treatment, for the odour of skatol became very apparent when steam was allowed to escape from the autoclave. The recovered pigment no longer emitted the odour of burnt feathers on being incinerated, but the presence of nitrogen was nevertheless conclusively shown by Lassaigne's method. The pyrrol reaction could no longer be obtained from the vapours emitted on dry distillation. This last fact is of importance. The presence of much pyrrol among the products of dry distillation of the melanins has led certain chemists to suspect that these substances are pyrrol derivatives, and we ourselves at first held this view. In two trials with a pigment obtained as above, and in a third trial with a pigment that had been heated to 260° C. with a saturated solution of barium hydrate, we failed to get the pine-sliver reaction for pyrrol, although a far smaller amount of the original pigment gave the reaction unmistakably. The vapours given off on burning some of the pigment purified in the manner just described with barium hydrate are difficult to characterize; but the odour of burnt feathers and of pyrrol is certainly not present.

In the experiment just referred to, in which barium hydrate was used to free the pigment from the ground substance, 1 gramme of pigment was kept at 260° C. with a saturated solution of this agent for four hours. After such treatment, this specimen of pigment, as we have already noted, failed to give the pyrrol reaction; and, strange to say, it was almost entirely precipitated from its solutions in nitric acid on the addition of an alkali, even when this solution no longer contained any barium.

We believe that the last-named method furnishes us with a means of entirely removing the ground substance from the pigment, and it must be left to later research to show with its help whether the substance thus obtained is a single chemical individual.

The substance obtained when the pigment is heated under pressure with potassium hydrate is perhaps closely related to the hippomelaninic acid obtained by Berdez and Nencki,* by fusing with potassium hydrate the melanin isolated from melanotic tumours of the horse.

It will be seen that we are only at the beginning of a true chemical knowledge of the melanins of the skin and hair. It would seem very probable that the melanins thus far described by investigators were all more or less contaminated with the proteid to which we have referred. But it is certainly a great advantage to see our way to an easy removal of all contaminating substances, and we propose, with the help of this method, to continue our investigations into the chemical nature of the melanins of the skin and hair.

14. Combustion Analyses.

Below are given the results of combustion analyses of pigment from the skin and hair prepared in various ways. We are fully conscious of the fact that these analyses do not assist us in establishing an empirical formula for our pigment, for the reason that the true pigment is still contaminated with the proteid-like substance that constitutes the groundwork of the pigmentary granules. Our object in making them was to learn whether different methods of preparing the pigment and prolonged treatment with strong alkalies really alter the so-called isolated pigment, when neither colour nor chemical behaviour have been in the slightest degree affected. Had spectrophotometric methods been applied, it is probable that they would have supplemented these analyses in a valuable way, or even have rendered them unnecessary.

* *Arch. f. exp. Path. u. Pharmakol.*, Bd. xx, p. 359.

Pigment of the epidermis, prepared by repeated precipitation with alcohol and ether and a little acetic acid out of solutions in dilute potassium hydrate, and finally out of solutions in ammonia, from which, however, the ammonia had been expelled before precipitation by the agents named. The special feature of this method is the mild treatment with potassium hydrate. The pigments of both the epidermis and hair are excessively hygroscopic, so that all weighings must be performed in well-closed capsules.

0.2972 gramme of pigment burned in oxygen current with lead chromate, finely powdered lead chromate being also strewn on the substance in the combustion boat, gave 0.5468 gramme of CO_2 and 0.1000 gramme of H_2O. The ash content of this specimen, as determined by previous analysis, was 3.17 per cent; calculating for ash-free substance, we find that the above specimen contained 51.83 per cent C and 3.86 per cent H.

0.3656 gramme of substance gave by fusion with KOH and KNO_3, after previous oxidation with concentrated nitric acid—the analysis being carried out in detail according to the directions of Hammarsten *—0.0928 gramme of $BaSO_4$ = 3.60 per cent S, calculated for ash-free substance.

0.2065 gramme of substance gave by the method of Dumas 24.7 cubic centimetres of nitrogen with the barometer at 759.2 millimetres and the temperature 28.5° C. N = 0.02802 gramme, or, calculated for ash-free substance, 14.01 per cent.

Hair pigment prepared in the same manner as skin pigment, except that it was subjected for a longer time to treatment with potassium hydrate. 0.1922 gramme gave 0.0073 gramme ash = 3.79 per cent.

0.2439 gramme of pigment gave 0.4536 gramme of CO_2 and 0.0746 gramme of H_2O, or C = 52.74 per cent and H = 3.53 per cent.

0.8114 gramme of pigment gave 0.1897 gramme of $BaSO_4$, the analysis being carried out as already described for the sulphur analysis of the skin pigment. Calculated for ash-free substance, S = 3.34 per cent.

0.3953 gramme of substance gave by the method of Dumas 34.8 cubic centimetres of nitrogen, with the barometer at 760.2 millimetres and the temperature 26.5° C. N = 0.03995 gramme; correcting for ash, we find N = 10.51 per cent.

The percentage composition calculated for the ash-free pigment is therefore as follows:

* *Zeitschr. f. physiol. Chem.*, Bd. ix, p. 289.

Pigment of the Epidermis.		Pigment of the Hair.	
C =	51·83	C =	52·74
H =	3·86	H =	3·53
N =	14·01	N =	10·51
S =	3·60	S =	3·34
O =	26·70	O =	29·88
	100·00		100·00

It will be seen from the above table that the pigment of the epidermis differs markedly in its nitrogen content from that of the hair. This difference is very likely due to the more prolonged treatment with potassium hydrate. We have found that the method of preparation has a great influence on the percentage composition of the pigments, and it seems impossible to prepare two specimens from the same original material so that they will give the same analytical results. Thus, a specimen of pigment from the epidermis, analyzed in the early stages of purification by the alcohol method, had the following composition: C = 52.17 per cent. H = 4.14 per cent. N = 12.8 per cent. S = 4.02 per cent. O = 26.87 per cent.

The pigment of both skin and hair was next treated on the water bath with a very strong solution of the potassium hydrate, and the effect noted.

A quantity of pigment whose ash content had already been reduced to a low figure was dissolved in a ten-per-cent solution of potassium hydrate, and this solution was reduced in a nickel bowl on the water bath to fifty or sixty per cent in strength. Water was then added and the evaporation repeated, and this treatment was kept up for six hours. The solution was then diluted and filtered, and the pigment precipitated with an excess of acetic acid, after which it was collected on flat filters and washed with alcohol until all traces of potassium and acetic acid had been removed. The pigment was now taken up in ammonia, the ammonia expelled on the water bath, and the black solution filtered and precipitated with a large excess of alcohol and ether and a small amount of acetic acid. It was then collected on flat filters, washed free of acetic acid with alcohol, and then treated with hot alcohol, chloroform, and ether. Thus prepared, the pigment of the epidermis has the following composition:

0.1333 gramme of pigment from the epidermis gave 0.0011 gramme of ash, consisting of minute dark specks = 0.82 per cent.

0.1139 gramme of another specimen of pigment from the epidermis that had not been allowed to stand so long while in a state of solution gave 0.00142 gramme of ash, consisting of minute black specks = 1.24 per cent.

0.1570 gramme of pigment from the epidermis gave 0.3044 gramme of CO_2 and 0.0723 gramme of H_2O. Correcting for an ash content of 1.24 per cent, we have C = 53.56 per cent and H = 5.11 per cent.

0.2357 gramme of pigment from the epidermis gave 30.0 cubic centimetres of nitrogen, with the barometer at 767.4 millimetres and a temperature of 18° C.; correcting for ash, N = 15.47 per cent.

0.4126 gramme of pigment from the epidermis gave 0.0750 gramme of $BaSO_4$; correcting for ash, S = 2.53 per cent.

0.4170 gramme of pigment from the epidermis gave 0.07006 gramme of $BaSO_4$. This particular analysis was made with a specimen containing 2.34 per cent of ash. Correcting for ash, we have S = 2.36 per cent.

Pigment of hair treated with strong potassium hydrate as previously described:

0.2598 gramme gave 0.0057 gramme of a reddish ash = 2.19 per cent.

0.1665 gramme gave 0.34075 gramme of CO_2 and 0.0799 gramme of H_2O. C = 57.06 per cent; H = 5.45 per cent.

0.1646 gramme gave by the method of Dumas 17.6 cubic centimetres of nitrogen at 20° C., with the barometer at 758 millimetres. N = 12.87 per cent.

0.7337 gramme gave 0.0924 gramme of $BaSO_4$. S = 1.77 per cent.

Placing the results in parallel columns—

Pigment of Epidermis.		Pigment of Hair.	
C =	53·56	C =	57·06
H =	5·11	H =	5·45
N =	15·47	N =	12·87
S =	2·53 : 2·36	S =	1·77
O =	23·33	O =	22·85
	100·00		100·00

Comparing the later analyses—that is, after the prolonged digestion with strong potassium hydrate—with those previously obtained with the help of less destructive methods, we see that the sulphur content has been considerably lowered for both pigments, and the carbon hydrogen and nitrogen content raised.

We are inclined to believe that this change in the sulphur con-

tent of the pigments is due to the fact that the contaminating substance derived from the pigmentary granules has been in part decomposed by the potassium hydrate. The fact that it is impossible to isolate two specimens of the pigments so that their percentage composition does not vary with even slight differences in the method of preparation, also points to the conclusion that we are not yet dealing with a single chemical individual, that our so-called isolated pigment is a mixture of at least two substances, one of which is responsible for this variability. It seems very improbable that this variability can be referred to the pigmentary compound, for this retains its colour and its general chemical character, however severe the treatment to which it is subjected.

15. The Iron Content of the Isolated Pigments.

The pigmentary granule, as we have seen, contains much iron, but, as the pigment isolated from these granules is purified, we find that the iron content steadily diminishes until but the merest trace remains, only to be accurately estimated by the spectrophotometric method.* We must therefore conclude that iron is not present as a constituent part of the molecule of the pigment; that it does not enter into its chemical structure, as does the iron of hæmatin, for example, but is present only as an impurity.

The present diversity of opinion as to the origin and iron content of the melanins, of which our pigment is one, is, we must believe, owing to the fact that a clear distinction has not always been made between the complex pigmentary granule and the pigment itself. The former contains iron; the latter does not.

In support of our view as to the low iron content of the pigmentary substance, we present, first, analyses of pigment with a relatively high ash content, and then analyses showing the ash content reduced to a low limit.

Thus, a specimen of pigment prepared by precipitating its first solution in potassium hydrate with strong acetic acid, and then wash-

* Cf. Mörner, *Zeitschr. f. physiol. Chem.*, Bd. xi, p. 87.

ing the collected pigment thoroughly with this acid, had an ash content of 3.3 per cent and an iron content of 0.14 per cent, estimated by titration with a weak solution of potassium permanganate. We always found this high percentage of iron when the pigment had not been repeatedly dissolved and precipitated.

Another specimen which had been prepared by the method of frequently repeated precipitation with alcohol gave the following results: 0.3863 gramme yielded 0.0134 gramme of ash, or 3.46 per cent. The amount of this ash taken up by dilute hydrochloric acid, after first rendering the silicic acid insoluble, was 0.0079 gramme. The amount of iron in this solution, estimated by the gravimetric method of Ludwig and Gottlieb,* was found to be 0.000385 gramme (0.00055 Fe_2O_3 obtained). The iron content of the pigment in this case was therefore 0.099 per cent. The residue of the ash insoluble in dilute hydrochloric acid consisted for the most part of silicon dioxide, and weighed 0.0055 gramme. In this instance we see that the iron has been much reduced, although the ash remains high, on account of the alcohol method being used.

A still lower iron content is found in specimens of pigment that have been prepared by the method of prolonged treatment with potassium hydrate, as already explained.

I. Thus 0.2253 gramme yielded 0.00529 gramme of a black, powdery ash. Of this ash 0.00359 gramme passed into very dilute hydrochloric acid, after having first rendered the silicic acid insoluble. The iron of this amount was found by titration with a weak solution of permanganate to be 0.00017 gramme, and constituted therefore only 0.076 per cent of the pigment. The part of the ash insoluble in dilute HCl consisted of silicon dioxide and aluminium oxide.

II. Pigment = 0.1333 gramme, ash obtained = 0.0011 gramme, or 0.82 per cent, and containing 0.00003 gramme of iron, as estimated by colour titration with KCNS. This specimen of pigment therefore contained only 0.02 per cent of iron.

III. Pigment = 0.1139 gramme; ash obtained = 0.00142 gramme, or 1.2 per cent, and consisting of minute black specks with a fused appearance. The iron of this ash, as determined by titration with a weak

* *Arch. f. exp. Path. u. Pharmakol.*, Bd. xxvi, p. 139.

solution of permanganate, amounted to 0.00016 gramme, or 0.14 per cent of the amount of pigment taken.

The ash of the hair pigment was also analyzed as above described, and with similar results. When purified until a low ash content was obtained—say 2.19 per cent—the iron was also found to amount to far less than 0.1 per cent. Thus, 0.4492 gramme of substance gave 0.0078 gramme of ash, the iron of which, as determined by titration with weak permanganate, amounted to 0.00026 gramme, or 0.057 per cent.

The iron determinations recorded in this paper, both of the granules and of the pigment, were made in three ways, and we have usually indicated in each case which method was employed. When only a mere trace of iron was thought to be present, the method of analysis was by colour titrations with potassium sulphocyanide; the second method, the one most frequently employed, was by titration with a solution of potassium permanganate, each cubic centimetre of which was capable of oxidizing 0.00043 gramme of iron; the third was by the gravimetric method of Ludwig and Gottlieb, which has been found trustworthy up to the limit of half a milligramme of iron, when only small filters and small quantities of reagents are used. Thus, one of us (Abel) recovered by this method half a milligramme of iron from a few cubic centimetres of a solution containing precisely that amount of iron which had been standardized by Dr. T. B. Aldrich. Miss Katharine Porter also recovered by this method 0.00239 gramme of Fe_2O_3 out of 0.00244 gramme taken. The Fe_2O_3 was prepared from pure ferric chloride.

16. The Origin of the Pigmentary Substance.

When a pigment is found to contain iron, this fact has been thought to furnish some evidence that it is a derivative of hæmoglobin; but if no iron is present, it may still claim that source, for the animal organism contains a number of pigmentary substances, such as hæmatoidin and bilirubin, that have no iron in their composition, and which, nevertheless, are derived from hæmoglobin. This class of iron-free pigments, it will be remembered, lack the element sulphur.

The coloured substances of the epidermis and hair agree with this class in containing no iron, but they differ in that they contain a considerable amount of sulphur, which we must consider to be present as a constituent part of their molecule. We are not at present acquainted with any coloured natural derivative of hæmoglobin that contains sulphur, nor do we know of any such produced by chemical means. The proteid moiety of hæmoglobin contains but little more than a half per cent of sulphur, while our pigment contains at least three times this amount.

The presence of sulphur in the pigment makes it certain that it is derived from a proteid, and we can as easily suppose some proteid of the blood to be the direct precursor of the pigment as that hæmoglobin should be decomposed in order that its proteid moiety may serve this purpose. We should have an equal right to assert that keratin is derived from hæmoglobin.

17. Total Amount of Pigment and of Pigmentary Granules in the Skin of One Negro.

The entire skin, with the exception of that on the palms of the hands and soles of the feet, was removed from the cadaver of a tall, slender negro, and from this skin the epidermis was removed with the exception of a few small areas. After being cleansed with water, alcohol, and ether, the epidermis thus obtained was found to weigh 34.08 grammes. Making a liberal allowance for the thicker epidermis left on the feet and hands, and the small areas not removed from the knees and elbows, we may assume that the entire removable epidermis of the negro in question, when freed of hairs, fatty substances, water, etc., would have weighed 40 grammes. 9.9306 grammes of this epidermis yielded 0.2182 gramme of pigment, the pigment being gathered and weighed immediately after its first precipitation. Calculated on this basis, we find that the epidermis of this cadaver must have contained at least 0.8789 gramme of pigment. The individual in question was judged to be a full-blooded negro, but his colour was of the bronzed order rather than deep black. The epidermis was also thinner than usually seen, and the case may therefore be assumed to

contain somewhat less than the average amount of pigment. We therefore assume the average amount of the pigmentary substance, as at present isolated, to be in the neighbourhood of 1 gramme for an adult negro of average body surface. If we assume, also, that the pigmentary granules as lodged in the living cell contain sixty-five per cent of water and about five per cent of mineral constituents, there would be found in the skin of the average negro about 3.3 grammes of pigmentary granules.

18. Function of the Epidermal Pigment.

While we ourselves have done no work on the function of the pigment, the subject is one of such interest that we must be allowed a brief account of the investigations of others.

Hom * exposed his own hand alongside of that of a negro to the direct rays of the sun, with the result that his own hand became inflamed, while that of the negro remained unaffected, thus showing how great a protection is afforded by the pigment of the negro's skin. C. Eijkmann,† of Batavia, has recently published a most interesting experimental research on the regulation of body temperature among whites and Malays in Batavia. The relative ability of the two kinds of skin to radiate and conduct heat, and the extent to which sweating takes place in the two races, were carefully studied. By the following experiment the two kinds of skin were also compared as to their ability to absorb sunlight, in which respect they were found to differ markedly. Two thermometers exactly alike were treated as follows: The bulb of one was covered, first, with a piece of skin from a Malay, and outside this with a piece of skin from a white man. On the second thermometer the order of the skin wrappings was reversed, the white skin being put next the bulb and the brown skin outside. After a period of exposure in a moist chamber to the direct rays of the sun,

* Cited from Boubnoff, Ueber das Permeabilitätsverhältniss der Kleidungsstoffe zum chemisch wirkenden Sonnenstrahl. *Arch. f. Hyg.*, Bd. x, p. 363. This investigator's name is given as Horn by Eijkmann in Virchow's *Archiv*, Bd. cxl, p. 157.

† Virchow's *Archiv*, Bd. cxl, p. 125.

the first thermometer registered 47.5° C., the second 50.1° C. Eijkmann also says that the brown skin felt distinctly warmer to the hand than the white skin. Now Widmark * has shown that the ultra-violet rays of the spectrum are far more effective in causing that superficial inflammation known as sunburn (erythema solare) than are the rays of less rapid undulation. Indeed, sunburn and that more marked inflammation resulting from exposure to a very powerful electric arc light are both caused to so preponderating an extent by the ultra-violet rays that heat, the degree of illumination, and other factors that have hitherto been regarded as causative agents, may be entirely neglected.

Hammer † has furnished corroborative experiments by covering the skin with ointments and lotions containing sulphate of quinine, which has the power of changing violet rays and ultra-violet rays to less refrangible ones, and has thus succeeded in greatly lessening the inflammatory action of sunlight and of the arc lamp. Bouchard is also cited by Paul Bert ‡ as having demonstrated that violet rays are more effective in raising a blister than rays of less refrangibility. Hammer has also abstracted from the literature of dermatology a number of interesting cases of great sensitiveness of the skin to light, and in all of these cases it was shown that the chemically active rays alone were responsible for the inflamed condition of the skin.

It is a matter of common observation that the photograph of a negro is indistinct and unsatisfactory compared with that of a white man, the light reflected from the black skin having been largely deprived of its actinic quality. Boubnoff's # experiment with frog's skin placed over sensitive plates has also shown how effective are thoroughly pigmented areas in absorbing the chemically active rays of light. It may therefore be assumed that it is one function of the pigment to absorb the chemical energy of the sun's rays, and, although the pigmentary layer of the skin would thereby tend to take on a higher

* Cited at length in Hammer. Ueber d. Einfluss des Lichtes auf d. Haut. Stuttgart, 1891, pp. 29-33.

† *Op. cit.*, pp. 33-41.

‡ *Rev. scientifique*, 1878, No. 42, p. 987.

\# *Loc. cit.*, p. 362.

temperature, yet the inflammation of the more sensitive vascular corium underneath is in reality prevented.

19. Conclusions.

The pigmentary granules of the negro's skin and hair can be freed in several ways from the cells in which they are lodged and collected in any desired amount.

As thus obtained, these granules are found to be insoluble in dilute alkalies, dilute hydrochloric acid (hot or cold), alcohol, or other organic solvents when applied in the order named. If, after they have been subjected to the action of dilute hydrochloric acid, they are again treated with dilute alkalies, they are found to give up their pigment, and, on the continued application of heat, the granules dissolve entirely in the alkaline solution, leaving only an insignificant residue.

The pigmentary granules are composed of a colourless ground substance or substratum, a pigment, and much inorganic matter. Their inorganic constituents, as thus far determined, are calcium, magnesium, iron, and silicic, phosphoric, and sulphuric acids; and these constituents possibly play an important part in the deposition and fixation of the colouring matter in the granules.

The pigment isolated from the granules, and sufficiently freed from adherent inorganic matter, contains only the merest trace of iron—so little, in fact, that we must think of it when entirely pure as free of iron. Heating the isolated pigment with barium hydrate at a temperature of 260° C. entirely frees it from the closely adherent ground substance, and it is then found that the vapours of pyrrol are no longer emitted when it is subjected to dry distillation, and the odour of burnt feathers is no longer discerned, although nitrogen is still present.

We can not conclude as the result of our work that the pigment is a derivative of hæmoglobin; it seems to us more probable that it is ultimately derived from the proteids of the parenchymatous juices.

The total quantity of soluble pigment in the skin of a negro of average size is found to weigh about 1 gramme; the weight of the pigmentary granules is about 3.3 grammes, if we are right in our assumption

that they contain sixty-five per cent of water and five per cent of mineral constituents in their natural state in the epidermis.

The pigments of the epidermis and hair of the negro are very likely identical. In the present state of our knowledge we can only say that it seems highly probable that the pigment of the negro's hair is not different from the dark pigment found in the hair of the white races, and we may infer that the pigment of the black skin differs only in amount and not in kind from that deposited in the skin of the white man.

Description of Plate XIV.

Fig. 1.—A single cell from the lowest stratum of the rete mucosum, showing the pigmentary granules.

Fig. 2.—The epidermis after maceration for two hours and a half with a five-per-cent solution of potassium hydrate.

Fig. 3.—The same after maceration for six hours. The pigmentary granules here seen have been spread out in a thin layer by slight pressure on the cover glass.

Fig. 4.—The dried pigmentary granules in the form of the snuff-coloured powder described in the text, mounted in water.

Fig. 5.—The pigmentary granules of the epidermis stained with Ehrlich's gentian-violet solution after having been boiled in a sealed tube with a mixture of concentrated ammonia and alcohol.

Fig. 6.—A piece of hair macerated for fifteen minutes in five-per-cent potassium hydrate and then crushed and mounted. It shows the large ovoidal pigmentary granules.

Fig. 7.—The isolated pigmentary granules of the hair stained with Ehrlich's gentian-violet solution, after having been partially decolourized by means of concentrated ammonia and alcohol.

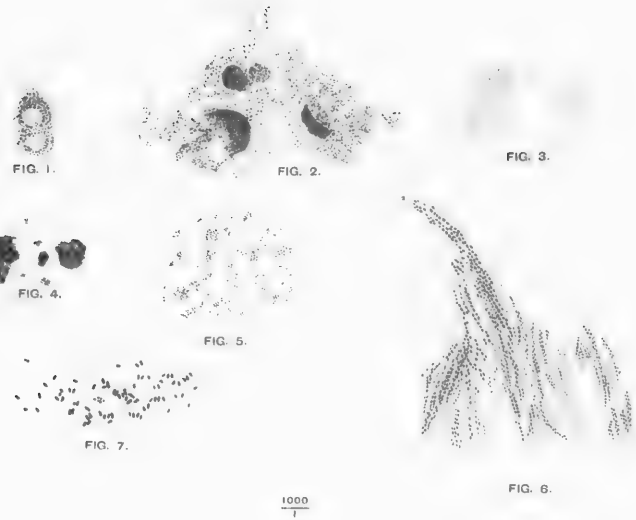

FIG. 1. FIG. 2. FIG. 3.

FIG. 4. FIG. 5.

FIG. 7. FIG. 6.

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ON HÆMORRHAGIC CYSTS OF THE THYROID GLAND.

By W. I. BRADLEY, B. A., M. D., M. R. C. S.

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PLATE XV.

It is generally taught that the most frequent form of enlargement of the thyroid gland is the parenchymatous goitre, or bronchocele, a form in which, by the overproduction or retention of colloid material within the vesicles in general, there is brought about a very considerable increase in the size of one lobe or in that of the whole organ. There is, however, another form of cystic enlargement, not generalized, but localized, in which the enlargement is due not to multiple retention cysts, but to the development of large isolated, cystic swellings, either solitary or not exceeding three or four in number. The size of these cysts greatly exceeds that of the individual distended follicles in the most extreme cases of parenchymatous goitre, and the walls as well as the contents are of a wholly different nature.

Judging from the material which I have had the opportunity of studying, this in Canada constitutes quite the most frequent form of unilateral and considerable enlargement of the thyroid presenting itself for operation. Out of eighteen cases of bronchocele operated upon by Dr. Shepherd at the General Hospital in Montreal, the material from eight of which came into my hands, no less than nine, if not ten, were of this nature,* a proportion much larger than I had been prepared to encounter. A study of the literature bearing upon the morbid histology of the gland has convinced me that, relatively, very little attention has been paid to the nature and characters of these

* Of these eighteen, one was a case of generalised parenchymatous goitre, four were definitely "colloid cysts" (adenomatous nodules with retention), nine were definitely of the type here described, the remaining four being of doubtful nature.

large cysts; and, though it may be true that elsewhere they do not form so large a percentage of the cases of goitre operated upon as they do here in Dr. Shepherd's practice, nevertheless since surgical literature shows that they are very common, and now that operative interference with the thyroid is becoming increasingly frequent and increasingly successful, it may be opportune to draw more attention to their structure and characters than has hitherto been accorded to them.

Dr. Shepherd has described the surgical features of his cases in detail in the *Annals of Surgery*.* It will, therefore, be only necessary to append here a table of the cases, giving a brief epitome of the main points in connection with each.

Case.	Name.	Age.	Size, position, and contents of tumour.	Duration.
I.	Mrs. S.	56	Two cysts in the right lobe—one the size of a Tangierine orange, the other that of a walnut. *Contents:* a thick, reddish-brown material.	Ten years ago, when crossing the Atlantic, she was violently sea-sick. Lump on right side of neck was noticed shortly afterward. This continued to grow steadily, and had increased in size considerably during the last thirteen months.
II.	Mrs. G.	25	Two cysts in right lobe, the larger more superficial. *Contents:* a thick, greenish-brown fluid.	Noticed for six years. Growth at first small, slowly increased until a year before operation; then grew rapidly, the position becoming somewhat altered.
III.	Miss L.	23	Solitary cyst the size of a lemon in the right lobe. *Contents:* grumous, containing cholesterine crystals; extensive papillomatous projections into cyst.	Present for three years; very slow enlargement until a year before operation, when there was a sudden increase in size.
IV.	Miss C.	21	Solitary cyst the size of a hen's egg in the left lobe. *Contents:* dark, yellowish-brown fluid with clots of blood.	Gradual enlargement noticed for a year; then sudden increase in size three months before operation.
V.	Mrs. J.	30	Solitary cyst the size of a small orange in the left lobe. *Contents:* straw-coloured fluid.	Noticed for twelve years.
VI.	Miss McL.†	19	Single enormous cyst the size of a turnip in the left lobe. *Contents:* dark brown, thickish fluid.	Present for six years; more rapid enlargement a few months before operation.
VII.	Miss McP.†	19	Large solitary cyst the size of a grape-fruit in the right lobe, extending beneath the sternum. *Contents:* dark-yellow fluid.	Two years; had enlarged rapidly during the six months preceding operation.

* F. J. Shepherd, *Annals of Surgery*, September, 1895.

† Cases VI and VII were operated upon shortly after Dr. Shepherd's article in the *Annals of Surgery* had gone to press, and consequently are not referred to in that article.

Before speaking generally upon the subject of these cysts, it will be advisable to record briefly the results of the microscopical examination in each case:

Case I.—In this there were two cysts in the right lobe, whose contents were reported by Dr. Shepherd to be of the nature of a reddish-brown fluid. The cyst wall did not come into my possession, the material supplied to me being an ingrowth into the cyst. It presented the characters of somewhat modified glandular tissue; the average diameter of the vesicles appeared to be 15 μ, the largest measuring 60 μ.

Owing to the great variation in the size of the follicles, it is only possible to give a rough approximate statement of the average size of the vesicles. Although these figures have been reached by estimating the diameters of fifty or more neighbouring follicles, it is not pretended that they represent more than a general approximation to the average size.

The majority of the collections of gland cells had no lumen, and were simply clumps of nucleated cells. Some of the vesicles contained a dense colloid material, deeply stained as though by blood pigment, and here and there the colloid masses showed a definite concentric appearance. In some, in the middle of the colloid, it was possible to recognise one or more clear transparent crystals, varying in shape from a square to an oblong, and in longitudinal diameter from 10 μ to 55 μ (Plate XV, Fig. 1). As to the exact nature of these crystals, I am not in a position to make any statement; they were unaffected by all the ordinary media employed in hardening and fixation, such as alcohol, Müller's fluid, and the essential oils. It must be added that the crystals were only present where there was surrounding interstitial hæmorrhage, with a degenerated condition of the epithelium.

Corresponding with the irregularity in the size and contents of the vesicles, their epithelium showed great variation in depth. There was periarteritis and also extravesicular (interstitial) hæmorrhages; the capillaries were dilated, and the vesicles widely separated. This presence of dilated capillaries and of hæmorrhages is worthy of note in connection with the reddish-brown character of the fluid contained in the cyst.

The above case is unsatisfactory to this extent, that the relationship of this intracystic growth to the cyst wall could not be made out,

and because the absence of the cyst wall prevented a fuller study of the case. In the remaining cases the material sent fortunately included the cyst walls, and thus gave a far more satisfactory insight into the nature of the cysts themselves.

Case II.—(Two cysts of the right lobe, the second removed through the posterior wall of the cavity left by removal of the first.)

The cyst wall in this case consisted of several irregular overlapping and not perfectly concentric layers of dense fibrous tissue, with interspersed occasional short imperfect layers, characterized by the presence of numerous small nuclei. Some of the outermost of these imperfect nucleated layers appeared to merge into more perfect gland tissue; they represented evidently the remains of atrophied lobules or collections of vesicles. Upon the inner surface of the cyst, section showed here and there accumulations of well-developed vesicles 50 μ to 200 μ in diameter, possessing a somewhat flattened epithelium; here and there several vesicles appeared to have run together into a common chamber.

There were, in addition, small areas of fibrosis and of calcareous deposits. The fluid filling the main cyst contained cholesterine crystals and large, round cells filled with fatty globules. The fluid had a glairy, yellowish-brown appearance.

Case III.—(Single cyst of right lobe.)

As in the case last mentioned, the cyst wall was formed of several layers of fibrous tissue, with intermediate partial layers of atrophied gland tissue. From the wall there projected inward large, firm masses of gland tissue, with vesicles varying in diameter between 50 μ and 400 μ, having a thin, low epithelium and very little tissue between the individual vesicles. The fluid contents of the cyst were grumous, and contained cholesterine crystals.

Case IV.—(Single cyst of left lobe, the size of a hen's egg.)

The cyst wall possessed the same features as those in the last two cases, except that it was not quite so dense, and that here and there in it could be observed flattened masses of colloid. Greatly elongated vesicles could also be seen gradually losing their epithelium, and passing into what might possibly be lymph channels (vide Plate XV, Fig. 4). Both on the outer and on the inner side of the cyst wall there was typical gland tissue, the diameter of the vesicles varying between 40 μ and 125 μ. In these masses of gland tissue protruding into the lumen of the

cyst were unmistakable signs of old hæmorrhages (deposits of pigment and cells containing pigment granules). The contents of this cyst were reported by Dr. Shepherd to consist of a yellowish-brown fluid, with blood clots.

Case V.—(Single cyst of left lobe, the size of a small orange.)

The wall of the cyst in this was much thicker than in the preceding case, and was almost entirely fibrous, though there were evidences of contained atrophied gland tissue. Externally, the gland tissue showed a chronic interstitial thyroiditis, and in places merged imperceptibly into the cyst wall. In addition, the capsule exhibited patches of calcareous degeneration; in the middle of one such patch the remains of an artery showing obliterating endarteritis could be distinguished. The papilloma-like ingrowths into the cyst showed dilated vesicles filled with a clear homogeneous colloid material; the vesicular epithelium was greatly flattened, and exhibited areas of atrophy with communication between the vesicles. There were the remains of old hæmorrhages with pigment deposits, and more recent hæmorrhages causing rupture of the vesicular epithelium, so that the blood was both intravesicular and extravesicular. The contents of the cyst were reported by Dr. Shepherd to consist of a straw-coloured fluid.

Case VI.—(Single enormous cyst of the left lobe of the size of a turnip.)

The wall of this cyst, which had of late grown rapidly, was comparatively thin, and in its immediate neighbourhood was found normal thyroid tissue, the vesicles averaging about 25 μ in diameter. All around the inner aspect of the wall was similar tissue in fair quantities forming an inner lining, and showing here and there a hæmorrhagic focus. At one place a large papillomatous mass projecting inward showed thyroid tissue of a similar nature, with, however, much fibrous tissue and great compression of the vesicles. According to Dr. Shepherd's report, the contents consisted of a dark-brown, thickish fluid, and from the posterior wall there projected several papilloma-like patches the size of walnuts.

Case VII.—(Large soft cyst of right lobe, extending beneath the sternum over the vessels arising from the arch of the aorta; the cyst in consequence could not be shelled out in its entirety.)

The wall of the cyst was very similar to that of the preceding case. From the inner aspect there was thyroid tissue projecting, with vesi-

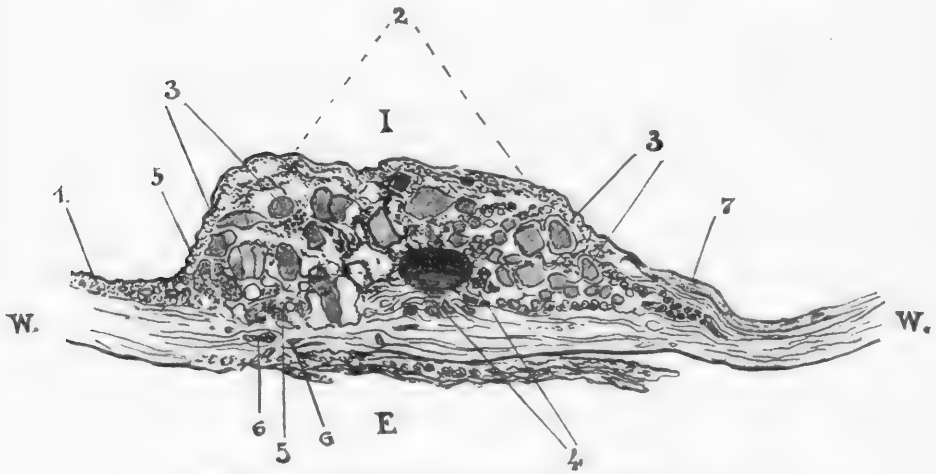
cles generally large, varying from 40 μ to 250 μ, the average diameter being calculated to be about 75 μ. Here the interstitial fibrous tissue was much increased, the colloid in the follicles was shrunken, and there were marked evidences of multiple hæmorrhages, both intravesicular and extravesicular. The contents of the cyst were reported by Dr. Shepherd to consist of a dark-yellow fluid. One large mass of gland tissue projected from the posterior or internal wall.

Case VIII.—The eighth specimen received from Dr. Shepherd was of a different nature. While at the operation the case appeared to be one of multiple large cysts with colloid contents affecting both lobes, upon microscopic examination of the dense colloid material enucleated, it was found that this was made up of thyroid tissue, with vesicles ranging from 25 μ up to 630 μ in diameter, the average being about 50 μ. The vesicular epithelium was greatly flattened, and consisted of little more than small rounded nuclei. Thus the case was one of multiple nodular or lobular overgrowths, or, more correctly, aberrant growths of the gland tissue. I have since received a similar specimen removed by Dr. J. Bell from a patient in this hospital.

Taking into consideration all of the various features presented by these large fluid cysts, it will be seen that the contents differ markedly from those of the ordinary vesicles of the thyroid gland. They are fluid, and in general present evidences of containing blood or derivatives from the blood; they vary from a straw-coloured fluid (as in Case V), through greenish brown (Case I), to a dark-brown, grumous fluid (as in Case VI); or, again (as in Case IV), actual blood clots may be present. It is of importance to note that when, as in Case VI, the growth immediately before the operation had been rapid and the cyst wall was thin, there was found the most abundant evidence of recent effusion of blood; when, on the other hand, as in Case V, the wall was thick and, judging from the calcareous deposits within it, the cyst was of long standing and quiescent, the contents showed least evidence of admixture with blood, consisting simply of a thin straw-coloured fluid.

With regard to the nature of the cyst wall, this is of a somewhat peculiar character. Although very definitely fibrous in composition, its nature is not sharply defined. The layers composing it are not

truly concentric; here and there between them occur masses of small cells, which, by comparison with the tissue immediately outside the wall, are seen to be clearly the atrophied remains of gland tissue (vide Plate XV, Fig. 4). These features explain why it is that in enucleation the cysts are found not to be sharply defined from their surroundings, and permit successive irregular layers to be partially peeled off. The wall, in fact, passes gradually into the bands of interstitial tissue running between the surrounding collections of vesicles, and



Portion of the capsule of the thyroid cyst removed from Case V. × 10.

E, external aspect; I, internal aspect; W, W, cyst wall of varying thickness. 1, Fine layer of thyroid tissue covering inner wall of cyst; 2, 2, mass of thyroid tissue projecting into lumen of cyst; 3, 3, large and healthy thyroid vesicles; 4, dense fibroid mass (atheromatous) with some calcification; 5, 5, small areas of hæmorrhage with pigmentation; 6, atrophied glandular tissue included between the layers of the cyst wall; 7, fibrous band or layer passing from the cyst wall to partially cover the projecting mass of thyroid tissue.

evidently represents not so much a new formation of fibrous tissue around the cyst as a compression of the surrounding thyroid tissue. In the older cysts, judging from the thickness of the wall, it would appear that there had been a certain amount of new fibrous tissue formation.

Upon its inner aspect also the wall differs widely from that of

an ordinary retention cyst; it is not lined either with well-developed epithelium or with the remains of such tissue, while between the contained fluid and the fibrous envelope is to be found irregularly distributed a greater or less amount of intact gland tissue.

The appearances here presented differ widely from what is to be observed in the ordinary parenchymatous goitre. In the latter the cysts are generally distributed through the substance, and, while individual cysts are often clearly recognisable with the naked eye, they do not attain to any great size. It is easy to follow the stages in their formation—that is to say, an examination of sections taken from cases of parenchymatous or colloid goitre reveals a series of modifications of the vesicles which can not but represent successive stages of the process. In the first place, the vesicles in general may be enlarged and distended with colloid material; later, as is well shown in Wölfler's figures of injected thyroids, where two distended vesicles are in close apposition, the vessels in between become compressed, and become atrophied—a result which is followed by atrophy of the neighbouring epithelium and of the interstitial substance. The next change is in all respects comparable with what obtains in the emphysematous lung under similar conditions. The atrophied tissue becomes completely absorbed, and the two vesicles fuse into one, small projections being left jutting into the common cavity, and indicating the site of the previous partition. This process of fusion may continue so that finally numerous vesicles may thus form one common chamber. The characters of such cysts are constant; they have a clear, sharply cut wall, covered by a continuous layer of epithelium, which is more or less flattened in different cases, according to the amount of pressure exerted by the contents. The walls of the cysts outside the epithelium are thin and indefinite. They are comparable, on the one hand, with the retention cysts to be met with in the various acinous glands—in the kidney, the mamma, the pancreas, and the liver—and, on the other, as above stated, with areas in the emphysematous lung. As with other retention cysts, in these there may occur secondary papillomatous ingrowths.

The whole appearance of the large cysts here referred to is that

of an accumulation in a lobule of the thyroid, the accumulation being associated with some destruction of gland tissue; not all the gland tissue of the lobule has been destroyed; some is left, and it is this that forms the characteristic irregular coating of the internal wall of the cyst. In support of this opinion is the fact that the large masses frequently found projecting into the cysts most commonly spring from the inner or medial aspect of the cyst, from the region where the lobules are not sharply cut off the one from the other.

It must be remembered that the thyroid is an imperfectly lobulated organ; toward the periphery the gland substance is seen to be partitioned off by fibrous septa, running in somewhat irregularly from the surface. These septa, however, become broken up and indefinite in the more central and deeper areas of the organ. But, apart from the character of the lobulation of the gland, it is to be noticed that in any case of accumulation of fluid in the interstices of the thyroid the tendency would evidently obtain for destruction and rupture of the tissue to occur in an outward direction—in the direction of least resistance. Thus, any advancing destruction of thyroid tissue would be most marked toward the exterior.

If this be so, and if these cysts be due primarily to destruction of thyroid tissue and accumulation of fluid in a space bordered by destroyed or partially destroyed vesicles, what is the cause at work leading to such destruction?

According to Wölfler, the main cause is an oversecretion of colloid in the vesicles, with consequent rupture of these, infiltration of the interstitial substance with colloid material, atrophy of the infiltrated tissue, and subsequent continued excretion of colloid from such of the epithelial cells of the ruptured vesicles as remain undestroyed. He devotes two pages to a consideration of the successive steps in such a process.*

So far as I have been able to determine, this view has been generally accepted, and no other explanation has been brought forward by more recent workers to explain the development of these cysts.

* Wölfler, Ueber die Entwickelung und den Bau des Kropfes, Berlin, 1883, p. 192.

Among recent French writers, Rivière,* in a treatise of one hundred and fifty pages, while discussing various diseases of the gland and pointing out that hæmorrhage into the vesicles may lead to a true hæmatocele, has nothing further to say with regard to the relationship between such hæmorrhages and cyst formation beyond the bare notice of the occurrence of hæmorrhagic cysts, and of the liability on the part of intra-acinous papillary growths to give rise to intra-cystic hæmorrhage. In English and American literature, descriptions of the removal of gross solitary cysts of the gland, evidently similar to those about to be described, are not infrequent, but the surgical features are in general dwelt upon to the entire exclusion of the histological characters. The only full and exact description that I have met with in English or American literature is in an article by Dalziel upon a case described as one of cystic adenoma.† His description tallies entirely with that given by me, and he is careful to draw attention to the very extensive hæmorrhages both into and around the vesicles, and to the evidence that in part, at least, these are of old standing.

But it would seem that against the theory of Wölfler there is not a little to be said. Rupture of a secreting organ or of the follicles of a secreting organ as a result of overactivity is most rare, even in the acini of glands which possess efferent ducts. When these ducts are obstructed, and retention cysts developed, the (at times) enormous expansion of the secreting surface in the cysts does not lead to spontaneous rupture. This certainly may be laid down as a rule to which there exist only peculiarly rare exceptions. Wölfler states that, in the thyroid, areas of such bursting of the vesicles can be recognised. While I am most ready to admit that he has examined a very much larger number of thyroids than has come into my hands, I feel it right to say that in more than twenty-five separate specimens of this organ which I have recently studied, the majority presenting one or other morbid condition, I have not seen any sign of this bursting of dilated vesicles, although I have carefully sought for it; or, to state

* Rivière, Contribution à l'étude anatomique du corps thyroïde et des goîtres, Lyons, 1893.

† Dalziel, *Glasgow Medical Journal*, xli, 1894, p. 227.

the case more exactly, where I have found hæmorrhage in the thyroid, with associated rupture of the vesicles, I have been unable to determine that this rupture was at the same time primary and spontaneous, associated with extreme distention.

Again, the nature of the contents of these cysts furnishes a strong argument in opposition to the above theory. If these were essentially the specific secretion from such of the epithelial cells of ruptured vesicles as remain undestroyed, we should expect to find them characteristically thick and colloid. But this is not the case. The peculiarity of the contents consists in the presence of cholesterine, of leucocytes loaded with fatty globules, and of a colour ranging from pale brownish yellow to the pronounced purple of hæmorrhagic effusion. The contents, it is true, are in general glairy, viscid, and albuminous, and contain, in all probability, some colloidal material; but this is greatly diluted, and altogether the specific secretion contained in the fluid appears to be of secondary importance. The so-called colloid cysts of the thyroid, from which upon enucleation thick, clear, semisolid matter is removed, are of a totally different nature from the cases to which here I would draw attention. According to my own observations upon two such "colloid cysts" (to one of which I have already referred), and the notes of a third case recently occurring under Dr. Bell at the Royal Victoria Hospital and reported to me by Dr. Adami, hardening and section of such enucleated colloid material show that it is not homogeneous, but is composed of numerous greatly distended vesicles with very thin walls. The material is, in fact, modified thyroid tissue, and the condition is one of cyst-adenoma, with localized overgrowth and retention of the colloid within the vesicles.

The examination of a series of thyroid glands obtained both from the post-mortem room and from the operation theatre has made very evident to me the frequency with which these organs become the seat of hæmorrhage. In case after case received from Dr. Shepherd and from Dr. Bell there have been indications not merely of recent hæmorrhages, which might have been accounted for by operative interference, but also of old extravasations of blood with the presence of

deposits of modified blood pigment, or again of large cells containing the characteristic modified hæmoglobin.

In Plate XV, Fig. 2, is shown one of these hæmorrhages. The section was obtained from the upper part of the left lobe of the thyroid of a patient who died in the Royal Victoria Hospital some weeks after drinking a large dose of potash lye, taken apparently with suicidal intent. In the region indicated was a roughly spherical tumour, a little over two centimetres in diameter, of comparatively firm consistence and of a rich red colour. Sections through the tumour showed that the hæmorrhage, which must have been comparatively recent, inasmuch as the red corpuscles were in general well preserved, was limited in extent to one lobule of the organ. Around it was a well-defined margin, formed of the connective tissue separating the lobule containing it from the surrounding lobules. In the affected lobule not all the tissue was the seat of hæmorrhage, sundry follicles and the surrounding interstitial tissue being entirely free from extravasated blood (Plate XV, Fig. 2, d). But, in general, there was clearly marked evidence of hæmorrhage. The blood had passed into the vesicles, and also into the interstitial substance. Many of the vesicles were very greatly distended with blood, and presented a thin, flattened epithelium; in one place an area was clearly distinguishable where there had been rupture of a small vessel, with a pouring out of blood both into the follicle and into the interstitial tissue. It was interesting to notice that in many places follicles distended with blood could be recognised, around which there was not the slightest sign of interstitial hæmorrhage. The appearance suggested strongly that the follicles communicate with each other, and that hæmorrhage had taken place into the follicles of one or more communicating systems, while other systems had remained free. Whether such communication is normal or acquired I will not venture to state. In several of the sections the communications between the injected follicles could be recognised; they were undistinguishable from what might have been produced by pressure atrophy, though, at the same time, natural and not acquired communications might present a similar appearance.

In this case, then, we have an example of a hæmorrhage limited to and distending one lobule of the thyroid and of rupture of the vessels resulting in distention of the follicles with interstitial disturbance. Of actual cyst formation the specimen showed no sign.

Plate XV, Fig. 3, represents a much further advanced condition. The case from which this was taken was one of parenchymatous goitre in a woman of twenty years, with diffuse hypertrophy of the organ most marked upon the right side. For four or five years the growth had been slow; during the last twelve months it had been more rapid.

Examined microscopically, the vesicles were found to vary greatly in size (from 15 μ to 300 μ in diameter, the average appearing to be from 25 μ to 30 μ). The larger vesicles were relatively rare, and occurred mainly in the deeper, more central portion of the gland. The contents of the vesicles had a shrunken appearance, and their epithelium was of well-formed columnar type. There was a certain amount of hyaline change in the interstitial tissue.

The enlarged gland presented several hæmorrhages, and upon a closer study of these under the microscope some vesicles were seen filled with blood, as in the previous case. There had, however, been more abundant extravasation into the interstitial substance, and, following upon this, a rupture of numerous follicles. In this way spaces had been produced filled with blood, and showing here and there along their edges layers of columnar epithelial cells, the remains of the burst follicles. Small collections of cells and masses of dislocated tissue could be recognised here and there in the blood-stained fluid, filling these rents in the thyroid. The hæmorrhages were not entirely recent, for in the interstitial substance and in some of the vesicles large pigment-containing cells could be seen.

Two series of changes might reasonably be expected to occur in connection with a lesion such as has been described. Either the epithelium of the ruptured follicles might undergo atrophy, the effused blood might become absorbed, and, through simple inflammatory and reparative processes, the lesion be eventually represented by a small mass of fibroid cicatricial tissue containing more or less blood pigment. In other regions of the specimen last described patches of

fibroid tissue of this nature were observed, and their presence would seem to indicate that small hæmorrhages had been frequent in this case. Or, on the other hand, where the hæmorrhage has been very extensive, there might be developed in the thyroid, as in the brain, a hæmorrhagic cyst, the contents of which, according to the age of the cyst, might, as in other hæmorrhagic cysts, vary from almost pure blood, with some admixture of the destroyed tissue of the part, to a straw-coloured fluid.

Such would seem to be the nature of the cysts here described. The two would possess features in all respects similar. Hæmorrhagic cysts of the nature I have indicated would tend to be limited by the interlobular connective tissue; within the boundary wall there would be not only fluid contents, but a lining of glandular tissue representing those portions of the lobule not destroyed at the time of the original hæmorrhage, and gaining a collateral circulation from the vessels of surrounding lobules.

Whether or not the remaining epithelium of the burst follicles continue to be well nourished and to pour out its excretion is a matter of possible doubt. For myself, I have never in a well-developed cyst seen the slightest indication of even a localized presence of an internal layer of thyroid epithelium; on the other hand, it must be acknowledged that the contents are at times very glairy, and strongly suggest some admixture of dilute colloid material. I regret to say that I did not at the time test the contents of any of the cysts for the presence of colloidal bodies.

On the theory of rupture and destruction of thyroid tissue, there is an anatomical basis for the frequent presence of large masses of thyroid tissue projecting into the cyst from its medial aspect. To this must be added the fact that the macroscopical and microscopical appearances of these ingrowths are wholly against the supposition that they are neoplastic; their surface is covered with a fairly dense layer of fibrous tissue, continuous with the internal layers of the cyst wall; their peduncles are, in general, broad, and pass imperceptibly into a layer of tissue containing thyroid vesicles, which, on the one hand, merges into the layer of glandular tissue lining the inner surface of

the cyst wall, and, on the other hand, did not in any specimen examined by me appear to be sharply differentiated from the surrounding and deeper thyroid tissue; in short, the vesicles in these papillæ resemble the vesicles of the tissue surrounding the cyst. I have already pointed out the many ways in which the contents of these cysts differ from ordinary thyroid secretion, and approach in character to hæmorrhagic products.

I am led, therefore, to regard these large solitary or rare cysts of the thyroid gland as being hæmorrhagic in origin, and I would speak of them as hæmorrhagic or post-hæmorrhagic cysts of the organ.

Many circumstances combined would seem to render the thyroid gland an organ peculiarly liable to be the seat of hæmorrhages. There is, in the first place, the peculiarly vascular character of the organ; in the second, its relatively exposed situation; and, in the third, there may be mentioned, as tending to explain the greater frequency of hæmorrhages, both small and large, in this organ in the female, the changes, apparently to a large extent vascular, which occur in connection with sexual disturbances. It is, however, clear, from the description given to me by Dr. Shepherd and recorded by him in his article in the *Annals of Surgery*, that in most, if not all, of the cases these gross cysts do not develop in healthy thyroids, but that there would seem to have been already some pre-existing morbid condition of the gland predisposing to their production. Judging from the portions of tissue surrounding the cysts that were sent to me, the condition most frequently associated with their development is that of a more or less advanced parenchymatous goitre.

As I have pointed out, in the parenchymatous goitre, even if there be no definite cyst production of a gross type, there may be numerous hæmorrhages; and when it is remembered how delicate are the walls of the distended cysts in this condition, and how abundant at the same time is the vascular supply of the organ and the network of capillaries immediately surrounding the distended vesicles, it is not difficult to realize why it is that such hæmorrhages occur, more especially when the relatively exposed situation of the enlarged gland is taken into

account. Here, in short, exists the most favourable combination of circumstances for the production of extensive hæmorrhages and of hæmorrhagic cysts of the thyroid.

There is one feature of these large cysts which is certainly difficult to explain. I refer to the frequent history given of progressive enlargement, more especially in the early stages. Unfortunately, the clinical histories of my cases are not of a detailed nature. In only one case is it definitely stated that the tumour appeared with comparative suddenness—after violent seasickness—and, when once developed, the patients have yielded no definite information as to whether the earlier growth was gradual, periodic, or irregular. In the later stages, in five out of the seven histories, it is noted that there was sudden or rapid increase. The grumous and blood-stained contents would seem clearly to indicate that this later and sudden increase in size is due to repeated hæmorrhages. In Cases II and VII the thyroid enlargement had been noticed for ten and six years respectively; nevertheless, the contents gave evidence of relatively recent effusion of blood. It is remarkable how frequent were the evidences of hæmorrhage in the projecting masses and surrounding tissue. Possibly the earlier growth is due to a like cause, but, as I have said, the clinical histories do not throw light upon this point.

The progressive enlargement can not be due to secretion from the epithelium lining ruptured vesicles, for in none of the cases of well-developed cysts have I encountered any sign of even localized epithelial lining.

It is possible that the cysts occur in and replace nodular parenchymatous hypertrophies or adenomata of the gland tissue, and that in some of the cases the progressive enlargement noticed in the earlier stages was due to the gradual growth of such nodules, which later became the seat of hæmorrhage and cyst formation. The material at my disposal, which consisted of the enucleated cysts and their contents, is incapable of deciding this question one way or the other.

That the growth, if gradual and cystic from the onset, is due to effusion or imbibitions of serous and colloid fluid from the surrounding vessels and lymphatics is but a remote possibility.

The most I can venture to state with any degree of certainty is that the more rapid and sudden enlargements of the cysts in the later stages can be ascribed to repeated hæmorrhages. I must leave to others possessing fuller opportunities the explanation of the nature of the gradual growth which, from the patients' reports, would seem to be the rule at the onset.

The view here enunciated may involve, it is true, only a slight modification of Wölfler's theory; nevertheless, it seems to me important to recognise the distinction between such spontaneous rupture of distended thyroid vesicles, as is urged by him, and the more inherently probable traumatic rupture with hæmorrhage, as here suggested.

Conclusions.

1. All the features peculiar to the gross cysts of the thyroid gland which possess fluid contents appear to indicate that they are essentially of hæmorrhagic origin.

2. While these gross cysts would seem more especially to occur in glands which already present the features of parenchymatous goitre, the theory of Wölfler that they originate as a consequence of spontaneous rupture of the vesicles is unsatisfactory.

3. It would seem more probable that these large cysts are due to rupture, traumatic or otherwise, of some of the vessels of the organ. The frequent signs of small hæmorrhages in cases of parenchymatous goitre without evidence of associated gross change in the surrounding vesicles, the structure of the organ and its exposed position, all appear to favour this view.

Finally, I beg to express my indebtedness to Dr. Shepherd for his kindness in affording me, while working mainly in the pathological department of the Royal Victoria Hospital, the material from the Montreal General Hospital, which has formed the groundwork of this paper. I beg to thank also Dr. James Bell for the rest of my material, and Prof. Adami, at whose suggestion I undertook this research, and to whom I am indebted for advice throughout its course.

Description of Plate XV.

Fig. 1.—From a hæmorrhagic area in one of the masses projecting into the cysts in Case I. Magnified 250 diameters.

The hæmorrhages in the projecting masses in this case were extensive but entirely interstitial, but the colloid contents, a a, of the vesicles had imbibed the blood-colouring matter and possessed a deep-brown colour, while in addition (in these hæmorrhagic areas only) there were to be seen the large intravesicular crystals, b b. The vesicular epithelium presented numerous fine vacuoles and other indications of degeneration. c c, blood effused between the vesicles.

Fig. 2.—From the interlobular hæmorrhage in the thyroid of Mrs. C. to show interstitial and intravesicular effusion of blood. Magnified 200 diameters.

a. Interstitial hæmorrhage. b. Vesicles filled with blood; disappearance of colloid material and very great distention, with flattening of epithelium. c. Region of rupture. d. Intact vesicles with colloid contents. f. Large pigmented wandering cells.

Fig. 3.—From an extensive hæmorrhage, very recent, in a case of parenchymatous goitre, Mrs. F., aged twenty years. Magnified 200 diameters. The figure shows rupture of the thyroid tissue. The effused blood is bordered in part by the epithelium of large ruptured vesicles, in part by interstitial tissue. (The former condition was only exceptionally observed in the sections.)

a. Intact vesicles of medium size. b. "Embryonal" masses of glandular tissue. c. Epithelium of large ruptured vesicles bordering upon the hæmorrhage. d. Broken-down thyroid tissue. f. Effused blood.

Fig. 4.—Section through the wall of one of the cysts, from Case IV. Magnified 250 diameters.

The section has been taken through a region where there was a low projection of thyroid tissue into the cyst, and the figure only includes the external portion of this and is not continued to the outermost portion of the cyst wall, where it passed into the surrounding thyroid tissue (beyond h).

a. Healthy vesicles lying along the inner wall of the cyst. b. Terminal portion of a long vesicle lying along the main fibrous tissue of the cyst wall. This contained blood, and, as shown in the figure here and there, along its outer aspect there was an absence of epithelium, this being replaced by a layer of flattened cells. c. Undeveloped gland tissue. d. Lymph space (?). f. Simple connective tissue forming the cyst wall. g. Undeveloped and compressed gland tissue in the thickness of the cyst wall. h. Lymph spaces, or vesicles that are atrophied and devoid of epithelium, filled with homogeneous colloid material. m. Corpuscles containing blood pigment. n. Epithelium of a vesicle tinged with blood pigment.

[All the figures have been drawn by means of a Zeiss's camera lucida. Fig. 3, taken from a somewhat thick section rich in deeply stained nuclei, while correct in its outlines, has its details rendered purposely in a slightly conventional manner.]

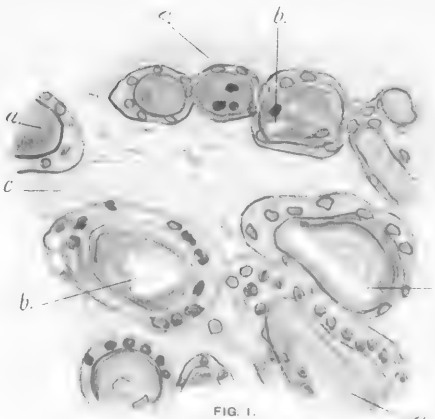


FIG. 1.

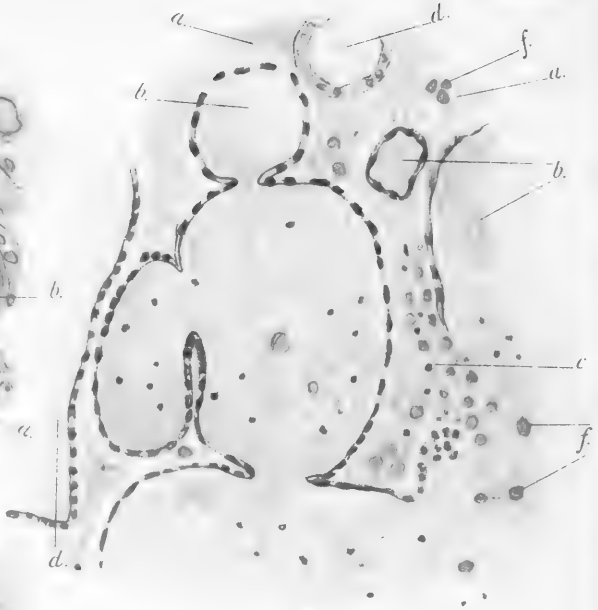


FIG. 2.

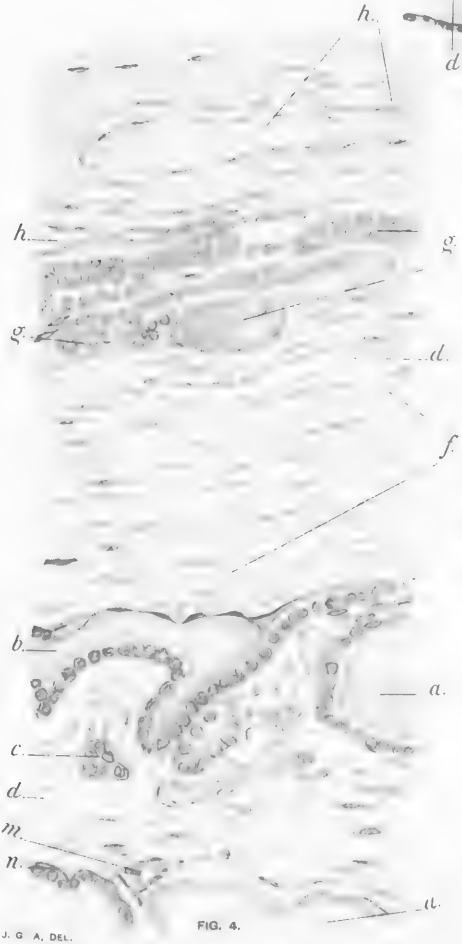


FIG. 4.

FIG. 3.

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THE SIGNIFICANCE OF PATHOGENIC SPIRILLA IN AMERICAN SURFACE WATERS, WITH A DESCRIPTION OF ONE ISOLATED FROM THE SCHUYLKILL RIVER AT PHILADELPHIA.

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PLATES XVI, XVII, AND XVIII.

SINCE the discovery by Koch of the spirillum of Asiatic cholera, many more or less similar spiral bacteria have been detected in materials and in places other than those closely connected with an outbreak of cholera. This is more especially the case since the recent epidemic in Hamburg.

Many of the vibrios that have been described and claimed to be similar to the cholera spirillum possess but few peculiarities that would lead to confusion on the part of one familiar with the characteristics of the genuine cholera organism.

On the other hand, a considerable number of forms have been detected in water, and in the stools of individuals suffering from other maladies, that possess so many of the characteristics of the true cholera spirillum that, without a specific reaction for this organism, their absolute differentiation is often a matter of extreme difficulty, and frequently of impossibility. In consequence, opinion has been divided as to the relation between the genuine comma bacillus of cholera and the organisms that simulate it; some believing them to be but different varieties of the same species, while others hold that they are definite and distinct species that resemble one another in only certain conspicuous group characteristics. There has therefore been for some time an active controversy between the supporters of these views.

The principal obstacle to the final settlement of this discussion seems to be removed by the discovery by Pfeiffer[1] of a specific relation between the serum of animals immunized from Asiatic cholera and the organism that causes the disease.

Pfeiffer has found that the serum of the blood of guinea-pigs that have been rendered immune from Asiatic cholera when injected into the peritoneal cavity of non-immunized animals affords protection to them against intraperitoneal inoculation of otherwise fatal doses of the cholera spirillum by inducing in the tissues of the animal into which it is injected a peculiar germicidal power through which the living organisms are actually disintegrated. He found further that if the animal thus treated be inoculated with vibrios that are sufficiently like the cholera spirillum to cause suspicion, but which are not genuine cholera spirilla, as may have been rendered probable or certain from other data, no such disintegration occurs, or if the animal be inoculated with a mixture of cholera spirilla and other similar spiral forms, only the cholera organisms are destroyed.

This important announcement has not thus far been refuted. On the other hand, it has met with abundant confirmation, especially through the investigations of Dunbar,[2] who has employed the test for diagnostic purposes upon a fairly large group of suspicious vibrios, and has found the results to accord with opinions previously formed from other data as to the nature of these organisms. From this standpoint, therefore, it seems that the differentiation between the several suspicious species and the genuine cholera spirillum can be made with certainty and comparative ease through the employment of the specific cholera serum.

More recently Pfeiffer and Vagedes[3] have described a microscopic reaction that occurs when the cholera spirillum is examined in a drop of serum from an animal very highly immunized from Asiatic cholera. This reaction consists in almost instantaneous cessation of motion on the part of the spirilla and their rapid conglomeration into masses. After twenty minutes in the incubator no morphological change is observed in the vibrios. After twenty-four hours the retarding influence of the serum seems to have disappeared and there is evi-

dence of development of the organisms. They state that the motility of only the cholera spirillum is influenced by this serum.

As has been already stated, the striking morphological, biological, and pathogenic resemblances between many of the cholera-like spirilla that have been found and the true cholera bacillus have led many to believe that they are nothing more or less than varieties or modifications of one and the same organism. The fact, as pointed out by Dunbar,[4] that the most suspicious forms have been found in waters of localities in which cholera had either existed or appeared subsequently lends a measure of support to this view; this, together with the observation that genuine cholera spirilla have occurred in these waters during the same seasons in which the other varieties were detected serves to further confirm the belief in the very close relationship between the several varieties. The finding of suspicious spirilla in the waters of western Europe in localities that have not been visited by cholera, though pointing to their lack of identity with the true cholera bacillus, does not positively contraindicate an identity under the conditions existing at the time of the discovery. We must bear in mind that during the past three or four years there have been numerous outbreaks of the disease at many points in that country, and one can readily conceive of the transportation of the genuine cholera spirillum from places in which cholera is epidemic to neighbouring localities free from the disease by means of water courses. While this may result in the production of cholera there is also justification for the opinion that this may occur without cholera being of necessity the result of such transportation, because of the modification in disease-producing properties that the organism may experience by its more or less long sojourn under the unfavourable conditions found in water.

It is plain, therefore, that while the test of Pfeiffer demonstrates a conspicuous difference between the genuine cholera bacillus and most of the important spirilla that are similar to it, still the view held by many with regard to their identity is supported by pretty strong arguments.

We must also not lose sight of the fact that vibrios have been found and described as cholera-like that were undoubtedly genuine

cholera spirilla that had undergone certain biological and pathogenic modifications.

Without entering into a review of the extensive literature on the subject (which is quoted in Dunbar's article[1]), it will suffice to say that the work of Pfeiffer in connection with the specific germicidal relation of the serum of animals immunized from cholera to the spirillum that causes this disease, and the absence of such relation to other similar spirilla, is thus far the only trustworthy contribution that has done aught to satisfactorily simplify this complicated problem.

An important disturbing element in all the European investigations has been the presence of cholera in the land, and in view of this it has occurred to me that it would be of importance to determine if suspicious spirilla are to be detected in the surface waters of countries that have not been visited, either recently or comparatively remotely, by Asiatic cholera, and which are sufficiently widely separated from localities in which the disease has been epidemic or is endemic to reasonably exclude the possible importation of genuine cholera bacilli by water or other ways.

Should investigations demonstrate the presence of suspicious forms in the waters of such places, it is certain that they can play no rôle in the production of cholera. It is also reasonably certain that they can bear no immediate relation to the organism causing cholera. Such a discovery, if made, would serve as additional evidence in favour of the want of identity between the cholera organism and at least some forms that simulate it in a sufficient number of important peculiarities to warrant their being classed as suspicious.

With this in view a series of investigations was begun in October, 1895, upon the more polluted portion of the Schuylkill River in its flow through the city of Philadelphia.

As the last visitation of cholera to this city was in 1873, when during the months of June, July, and August there were eight cases reported, two of which proved fatal, and about the nature of all of which there is room for doubt, the water of the river offers a favourable

field for such studies, especially as it is at this part of its flow grossly polluted with sewage.

As the result of studies thus far made, two spiral forms have been isolated, one of which is unimportant, as it possesses none of the group reactions common to the suspicious varieties, while the other is in every respect, morphological, biological, and pathogenic, sufficiently suggestive of the cholera group to be classed among the suspicious varieties and to warrant description at this time. The method employed for their isolation is that now universally employed in such work—viz., the modification of the method of Schottelius as recommended and applied by Koch.[5] It consists in the addition to 90 cubic centimetres of the water to be studied of 10 cubic centimetres of peptone-and-salt solution of ten times the usual strength employed in ordinary nutrient media, so that peptone is present in the entire mixture in the proportion of one per cent. The flask is then placed in the incubator at the temperature of 37° to 38° C. for from twelve to eighteen hours, when, without shaking it, gelatin plates are made from the surface of the fluid. If spiral forms are present they are usually abundant in the upper layers of the fluid, as may readily be seen by microscopic examination. In from twenty-four to forty-eight hours the gelatin plates, kept at from 18° to 20° C., are ready for examination. To one familiar with the appearance of the colonies of the cholera spirillum at this stage there is little difficulty in correctly anticipating the results of microscopic examination of colonies having a similar appearance.

By this means the organism about to be described has been isolated from the water of the Schuylkill and from the contents of a sewer emptying into it, the mouth of which is partly under water at high tide.

Pressure of work has prevented my making systematic studies upon this water with regard to the persistency of the spiral forms in it, so that I can not say at this time that they are always present, nor can I speak of seasonal variations. I hope, however, to obtain data on these points at some later time.

While it has been impossible to fully identify this organism with

any of the suspicious spirals described by European observers, still it simulates several of them so closely in certain important details that I am reasonably sure it is not a new species, but rather a variety.

For convenience of reference I propose to call it Vibrio Schuylkiliensis, though, I repeat, this is not to signify that it is considered as a distinct species.

Its characteristics are as follows:

Morphology.—It occurs in fresh cultures as short, rather plump "commas," often with a very decided curve, often less curved and often almost straight, though never quite so (see Photograph, Fig. 1). Its ends are usually rounded or slightly pointed. As usually seen, it is a little shorter and a trifle plumper than the cholera spirillum, though this is a very variable feature and means but little. There is no special tendency to grow into long threads, though occasionally spiral filaments, varying in length from three to five times that of a single comma, may be encountered. The single cells frequently join end to end to form S-shaped figures.

It is especially prone to undergo degeneration, and involution forms are often encountered in cultures as young as sixty to seventy-two hours. Such evidence of degeneration is especially conspicuous in potato cultures after forty-eight hours at 37° to 38° C. (see Photograph, Fig. 2).

It is actively motile, and depends for this property upon the presence of a single flagellum at one of its extremities. It does not form spores.

Staining.—It stains with the ordinary watery solutions of the aniline dyes; less readily with methylene blue than with either fuchsin or gentian violet.

It is decolourized by the method of Gram.

Very frequently the staining lacks uniformity. The single cells will present unstained portions; sometimes the poles only will be coloured, the centre being colourless; at times the distribution of the colour is more irregular and marked than at others. Fresh gelatin cultures stain somewhat more regularly than do agar cultures of the same age. Preparations made directly from the blood and tissues of

animals dead after inoculation with this organism usually show commas that stain irregularly.

Colonies on Gelatin.—In ten-per-cent nutrient gelatin rendered neutral or very slightly alkaline to phenolphtalein the colonies after twenty-four to thirty-six hours at 18° to 20° C. are fairly uniform in appearance, their outline being apparently dependent upon the number of colonies on the plate. For instance, in the "original" plate where large numbers are closely packed together they appear, when examined under the low power of the microscope, as very coarsely granular masses with no sharp border, presenting in general an appearance suggestive of minute chestnut burrs. On the plates in which there are fewer they are round, sharply defined, less coarsely granular, and have usually fine irregular markings as if they were creased or folded. Sometimes they present indistinct concentric markings. (See Fig. 3.)

As growth progresses these markings may become more and more distinct and finally give to the colony a decidedly lobulated or mulberry-like structure. This may continue without alteration until the lobulations are so marked that the appearance given in Photograph 8 results. At 18° to 19° C. this stage may be reached in from two and a half to three days. Or, from the condition noted at the end of twenty-four to thirty-six hours, the colony may become a trifle more granular and assume a distinctly concentric arrangement of zones, or a single zone surrounding a more or less uniformly dense and granular central mass, such, for instance, as is photographed in Fig. 6. This stage may be reached in from forty-eight to seventy-two hours at 18° to 20° C.

Again there may be only an imperfect arrangement of concentric zones; the central mass may be much more dense, with its circumference only partly defined and surrounded by a less dense zone, as is shown in Fig. 5.

Again colonies are encountered that stand, in so far as their structure is concerned, between the distinctly lobulated colonies and the distinctly concentric colonies, partaking in a measure of both characteristics. Such a colony is shown in Fig. 7.

At times lobulated colonies are seen, the lobulations of which are quite small, and very different in their arrangement from those shown in Fig. 8. Such a colony is seen in Fig. 9.

Less frequently colonies will appear that are finely granular from the beginning, and, beyond a few indistinct creases or ridges, take on no distinctive structure.

After about the third or fourth day, when liquefaction is actively in progress, the majority of the colonies lose their characteristic appearance. They are then seen as irregular, ragged, granular masses lying in the centre of pits of liquefied gelatin. These pits are usually surrounded by a tolerably definite peripheral zone that may be marked by microscopic, cilia-like processes, but which is more frequently irregularly granular at its edge, as is seen in Fig. 10.

To the naked eye the plates commonly have the appearance given by plates of the cholera spirillum—i. e., when quite young those containing many colonies have a ground-glass appearance. As growth continues, these plates are rapidly liquefied, usually completely so, in from thirty-six to forty hours. The plates containing fewer colonies are uniformly marked by the third day with sharply cut, funnel-shaped depressions filled with clear liquid, at the bottom of which the dense cream-coloured colony is seen. They exhibit no marked tendency to spread laterally, though sometimes one, sometimes more colonies will be observed on such a plate that spread laterally very rapidly, giving rise to a saucer-shaped area of liquefaction that strongly suggests the growth of colonies of Deneke's spirillum or that of Finkler and Prior.

Sometimes one or the other of the forms described above will predominate on a plate; equally as often both lobulated and concentric colonies will appear together. Occasionally all transitional forms may be detected, from the characteristic concentric to the distinctly lobulated or dahlia-like varieties seen in the photographs.

Once in a while, more especially when the gelatin has lost part of its density through being exposed to higher temperatures than usual, only an imperfect effort at characteristic colony configuration will be observed. Under these circumstances I have noticed colonies

having very much the appearance of those described and depicted by Ivanoff[6] for the colonies of the spirillum that bears his name—that is, they look as if they might be composed of convoluted threads.

From the description of Vibrio Metchnikovi as given by Pfeiffer,[7] together with the results of my own comparative studies of that organism, it is safe to say that in so far as the appearance of its colonies in gelatin is concerned, Vibrio Schuylkiliensis is practically identical with Vibrio Metchnikovi. As liquefaction becomes more and more advanced in the gelatin the plates give off a most unpleasant odour that it is impossible for me to describe. The reaction has now become excessively alkaline. A portion of this alkalinity is due to the presence of a volatile alkali which may readily be driven off by gentle heating, as may be detected by a bit of moistened litmus paper held over the plate. There is also an increase in the fixed alkalinity, for by higher heating, even boiling, the reaction is still seen to be much more alkaline than is the case with the uninoculated gelatin. By the addition of sulphuric acid to the liquefied gelatin in the plates a very bright, cherry-red colour is produced, evidently due to the presence of indol.

Gelatin Stab Cultures.—In the form of stab cultures in gelatin the appearance of the growth is essentially that of the cholera spirillum. It is usually a little more rapid, but at times is practically identical with it.

At the end of three and a half to four days there is the characteristic cup-shaped liquefaction, capped by the so-called air bubble. The growth continues downward as a fine line along the track made by the needle. In seven or eight days the relative proportions of this picture remain about the same, though the figure made by the growth has become magnified. In three weeks the characteristic appearance of the growth has disappeared. The gelatin in the tube is from one half to two thirds liquefied, the liquid is covered by a dense pellicle, and at the bottom is a dense cream-coloured mass that is often twisted or root-like. See Photograph, Fig. 11, a, b, c.)

Growth on Agar-agar.—On meat-infusion agar-agar containing two per cent of agar and neutral or slightly alkaline to phenol-

phtalein, growth is very rapid at body temperature. In twenty-four hours the colonies will range from 1.5 to 2 millimetres in diameter. They are not especially characteristic in appearance at this time, being simply smooth, lustrous, and opaque. After forty-eight to sixty hours they are usually more dry, occasionally a little wrinkled, with at times something of a concentric arrangement of wrinkled zones. The character of the growth on agar-agar varies with the conditions offered by the medium. For instance, in meat-infusion agar-agar rendered neutral or slightly alkaline to phenolphtalein (35 to 37 cubic centimetres of normal KOH to the litre) the growth after twenty-four hours at 37° to 38° C. is very voluminous, appearing as an opaque, smooth, moist glistening layer. After forty-eight hours it is drier, becomes more and more wrinkled, especially at the bottom of the tube, and often extends in the form of a dense pellicle over the water of condensation in the bottom of the tube. This pellicle is usually pushed a short distance up the sides of the tube. When one inspects this growth by the aid of a wire loop it is frequently found to be in its entirety a zoöglœa or pellicle that can be easily and cleanly stripped from the surface of the agar practically *en masse*. It holds together tenaciously and can be broken up in fluid only with trouble, and then not completely. As the culture becomes older the growth gradually takes on a dirty, coffee colour. These cultures give off a most pronounced odour of indol.

On agar-agar made from meat extract and rendered neutral to litmus paper the growth is much less voluminous and is not seen to adhere together in the zoöglœa-like way referred to above.

Blood Serum.—The growth of the spirillum on blood serum, made either according to the formula of Loeffler or in the ordinary way, after twenty-four hours at body temperature appears as a line of depression that does not tend to spread laterally. This continues until there is a distinct track of liquefaction marking the course of the development. As growth continues this becomes more and more conspicuous, and finally takes on a dirty, brown colour. The liquid that collects at the lower part of the tube is covered with a dense pellicle.

Bouillon.—Bouillon becomes uniformly clouded in twenty-four hours at body temperature. Its reaction becomes more alkaline as growth progresses. A pellicle, at first delicate, later denser, always characterizes the growth in this medium.

Potato.—On potato of normal reaction the growth after forty-eight hours at body temperature is very slight. At the point of inoculation there is a thin, glazed, more or less dirty yellow shading to a brownish deposit that is at times surrounded by a flat, dry, lustreless zone, but more frequently this zone is absent. On microscopic examination the organisms composing this growth are found to be commas in all stages of involution. As these cultures become very old (two weeks or more) the growth seems to have sunk slightly into the potato.

Litmus Milk.—In fresh milk containing a few drops of blue-litmus tincture a slight reddish tinge appears after twenty-four hours at body temperature. After forty-eight hours this red colour is usually increased, and at times there is coincident coagulation of the milk, though this is not always the case. There is but little increase of the red colour after the third day.

Peptone Solution.—In the ordinary one-per-cent peptone solution growth is rapid and is usually accompanied by a pellicle on the surface of the fluid.

After twenty-four hours at body temperature a brilliant indol reaction may be obtained in these cultures by the addition of sulphuric acid alone. A similar, though usually less intense, reaction may often be obtained under these circumstances after as short a time as six to eight hours at 37° to 38° C.

In peptone solution containing three or four drops of blue-litmus tincture to 10 cubic centimetres of the solution there is seen, after twenty-four hours at body temperature, an almost complete decoloration of the lower layers of the fluid. This extends no farther upward, as a rule, than the junction of the lower with the middle third of the solution. The upper part of the fluid retains its original blue colour. When such a culture is gently shaken the entire fluid becomes blue. After a week or ten days a dense pellicle is present

and the tone of the solution is somewhat greenish, or greenish blue. The colour of the solution never entirely disappears, though the lower decolourized zone is sometimes greater than at others. In peptone solution containing ten drops of rosolic-acid solution to 10 cubic centimetres of the medium there occurs, in addition to the pellicle formation, at first a slight diminution of the colour; this is followed by a low degree of intensification, and at the end of ten days the solution is usually of an orange-red colour.

Fermentation.—When cultivated in the fermentation tube in bouillon containing two per cent of glucose at body temperature, growth is abundant, though no fermentation with liberation of gas occurs.

Relation to Gases.—It is facultative in its relation to oxygen, though in an atmosphere devoid of this gas its growth is slower and less voluminous than when it has access to oxygen. This is a little less apparent with Buchner's pyrogallic-acid method than with the hydrogen method in sealed tubes.

In fluid media under an atmosphere of carbon dioxide in sealed tubes no growth is observed after seven or eight days at body temperature—probably because of the acidity of the medium due to absorption of the gas.

Relation to Temperature.—It grows most luxuriantly at from 37° to 38° C. Growth is hardly perceptible at 10° C. It is destroyed by an exposure of five minutes to 50° C., though it withstands this temperature for from one half to one minute.

Pathogenesis.—The pathogenic activities of this organism are most conspicuously seen in pigeons and guinea-pigs, both of which are uniformly susceptible to infection by it. In a few instances in which rabbits and grown chickens have been inoculated they have shown themselves to be naturally immune from relatively large doses. Mice may be infected with comparatively small doses by subcutaneous injection. If the dose be large they die within a few hours, evidently as a result of the toxic rather than the infective action of the material used.

In determining the pathogenic powers of this organism at the

time it was obtained from the river, it was soon manifest that positive results were to be obtained only through the employment of relatively large doses, as it was of comparatively low virulence. To meet this requirement a platinum-wire loop having a capacity of 6 milligrammes of a fresh agar culture was made, and with this loop the suspensions used have been made throughout the entire series of experiments. For convenience of reference I have designated a suspension made by finely dividing one of such loopfuls of a culture in 1 cubic centimetre of peptone solution as a " normal suspension "—and such will be understood when a normal suspension is referred to in the following part of this article. In only a few cases were fluid cultures used.

Inoculation of Pigeons.—When inoculated with the Vibrio Schuylkiliensis pigeons may or may not die. The circumstances that determine the results of inoculation are dose and mode of introducing the organisms. When doses of from 0.2 to 0.3 cubic centimetre of a normal suspension (equivalent to 1.2 to 1.8 milligramme of a solid culture) are injected into the pectoral muscle of pigeons death results in from sixteen to eighteen hours almost without exception, whereas if the solid culture be rubbed into a superficial wound of this muscle (a pocket beneath the deep fascia) in such large amounts as 2 to 4 milligrammes, only a portion of the animals succumb. For example: Of eighteen pigeons into the pectoral muscle of which normal suspensions of this organism were injected in doses ranging from 0.1 to 0.5 cubic centimetre (equal to 0.6 and 3 milligrammes of solid culture respectively), fifteen died and three recovered. Those that recovered had received 0.15, 0.25, and 0.3 cubic centimetre each (equal to 0.9, 1.5, and 1.8 milligramme of solid culture respectively). Of six pigeons into the pectoral muscle of each of which 1 cubic centimetre of a twenty-four-hour-old culture in peptone solution was injected, all died in less than seventeen hours.

Of eight pigeons inoculated by rubbing the solid culture into a superficial wound of the muscle below the deep fascia, only two died. Those that died had received 6 milligrammes each of the solid culture; of those that recovered, four had also received 6

milligrammes and two smaller doses—viz., 1.8 and 3 milligrammes, respectively.

On several occasions an attempt was made to infect pigeons by introducing into the muscle a bit of the necrotic muscle removed at autopsy from pigeons dead after this form of infection, and though relatively large pieces (about $\frac{1}{2}$ cubic centimetre) were introduced, death did not follow in a single instance. If, however, an infusion be made in peptone solution from a similar bit of necrotic muscle, and this be injected into the pectoral muscle of a pigeon, death follows with a fair degree of regularity.

When death ensues from either mode of inoculation the most conspicuous lesion found at autopsy is that localized about the site of inoculation. By superficial inspection the inoculated muscle is usually seen to be swollen, the skin over it being somewhat tense. On stripping back the skin an œdematous condition of the subcutaneous tissue is usually observed; at times this is very marked and may extend well down along the sides and over the thighs. Occasionally it is absent. The œdematous fluid is commonly of a bright-red colour. The diseased muscle is marked by parallel yellow striations that run in the direction of the muscle fibres. Sometimes these yellow lines will be lying together as a single narrow or broader zone that completely traverses the thorax, having, apparently, healthy tissue on either side of it. Frequently the entire muscle will be marked by these yellow lines, and in consequence will present a more or less uniformly pale appearance. On incising the muscle it is found to be mottled throughout with yellow and red necrotic areas; this condition extends down to the sternum and involves the underlying muscles. Fluid may or may not exude from the cut surfaces. By microscopic examination of cover slips from the necrotic area it is found to be everywhere invaded by comma-shaped vibrios in large numbers. The corresponding pectoral muscle on the opposite side is never found to be affected. It is normal in colour and size, even though the inoculated muscle may be markedly affected close up to the ridge of the sternum.

The heart is contracted, and is usually found to contain coagulated

or partly coagulated blood. The ventricles always contain much less blood than do the auricles. The lungs and kidneys are normal. At times the spleen may be a trifle large, though it is never markedly affected. The liver is dark in colour, sometimes mottled with diffuse lighter areas, but is otherwise normal.

The intestines show no departure from the normal as a rule; sometimes there may be one or two limited areas of injection.

By both microscopic and culture methods vibrios are found in very large numbers at and about the site of inoculation, practically everywhere in the diseased muscle. In fresh autopsies they are rarely numerous in the blood and internal organs, though they are to be found there in nearly all cases. For example: In fifteen autopsies, taken at random from my notes, the average number of colonies that developed from one loopful of blood from the heart was twenty-eight; in at least six other cases the number of colonies ran over two hundred, while in one or two instances only, the cultures from the blood were sterile. There is often such a small number in the blood that one may fail to find them by microscopic examination, though by culture methods they are, as is seen above, generally to be found.

At autopsies made some time after death—ten to fifteen hours—when the dead animal has been allowed to lie in a warm place the number of organisms in the blood is always greater, and if one places in the incubator the heart of a pigeon in which few or no vibrios are to be found microscopically, after a few hours the blood from it will be found to contain them in enormous numbers.

At fresh autopsies* the internal organs are also found to be relatively poor in bacteria.

The pathological lesions observed in pigeons dead after inoculation with this organism are so conspicuously similar in most of their important details to those resulting from inoculation with virulent cultures of Vibrio Metchnikovi that it is impossible to rid one's self

* When possible, these autopsies were made immediately after death. When this was not feasible the animals were placed either in the ice chest or at an equally low temperature as soon as they were found dead, and were kept there until the post-mortem examination could be made.

of the opinion that Vibrio Schuylkiliensis may perhaps be only an attenuated Vibrio Metchnikovi. To decide this point an effort was made to increase the virulence of the former organism, and though I have not as yet (up to April 23, 1896) been able to bring its virulence up to anything like the degree possessed by Vibrio Metchnikovi, it has still been increased to a point considerably beyond that given in the preceding statistics.

The method employed for increasing the virulence was that of passing the organism through a series of susceptible animals. After passing it through a series of twenty pigeons, each having been inoculated with the first generation on agar-agar from the tissues of a dead pigeon, its virulence was found to be about doubled when suspensions were employed, and about trebled when the solid culture was employed. Thus, for instance, from the twentieth pigeon of the series twelve pigeons were inoculated. Six received varying doses of a normal suspension, and six were inoculated by rubbing different amounts of the solid culture into a superficial wound of the pectoral muscle below the deep fascia. The results were as follows:

Pigeon	I	received	0·1	cubic centimetre.		Died in 27 hours.			
	II	"	"	"	"	Died in less than 11 hours.			
	III	"	0·2	"	"	"	"	20	"
	IV	"	"	"	"	"	"	20	"
	V	"	0·3	"	"	"	"	20	"
	VI	"	"	"	"	"	"	11	"

Results of inoculating pigeons with the same culture, but instead of injecting it as a suspension it was rubbed into a superficial wound of the pectoral muscle:

Pigeon	VII	received	2	milligrammes.	Had no effect.
	VIII	"	2	"	Died in less than 20 hours.
	IX	"	4	"	Died in 24 hours.
	X	"	4	"	Died in less than 20 hours.
	XI	"	6	"	Had no effect.
	XII	"	6	"	Died in less than 20 hours.

The injection of the normal suspension proved fatal, as we see, in all of the six pigeons, including those that received the dose of 0.1 cubic centimetre (equal to 0.6 milligramme of solid culture),

while of those that got the solid culture, four succumbed, death having resulted in one of these from the dose of 2 milligrammes. Though the doses used throughout this experiment are relatively large, still the smallest of them is smaller than any that had hitherto proved fatal to pigeons by the methods of inoculation that were employed. It does not seem unlikely, therefore, that by the further passage of Vibrio Schuylkiliensis through susceptible animals we may at last reach a still higher degree of virulence; in short, establish its pathogenic identity with Vibrio Metchnikovi, the organism with which it possesses so many other points in common.

Inoculation of Guinea-pigs.—Guinea-pigs are susceptible to the pathogenic activities of this organism by both subcutaneous and intraperitoneal inoculation.

By Subcutaneous Inoculation.—The fatal dose for guinea-pigs varies, according to the weight of the animal, from 0.3 to 0.5 cubic centimetre of a normal suspension of a twenty-four-hour-old agar culture. After inoculation with this dose death ensues in from twelve to eighteen hours.

At autopsy the tissues about the site of inoculation are much injected, and usually moderately œdematous. Frequently the subcutaneous œdema is very widespread, sometimes it is much less marked.

The subcutaneous lymphatic glands, both inguinal and axillary, are red and enlarged.

As a rule, there is more or less reddening of the peritonæum beneath the point of inoculation, with at times general peritonitis. This may, however, be absent. When present it is characterized by the accumulation of a small amount of fluid in the peritoneal cavity and by the presence of isolated patches of fibrino-purulent material scattered over the surface of the liver, spleen, omentum, and the coils of intestine. On microscopic examination this inflammatory deposit is found to be rich in leucocytes, to contain a few endothelial cells, and to be invaded by vibrios in large number.

As a rule the mucous membrane of the intestines is not inflamed, though once in a while they are found quite red and filled with dark, red-brown, semi-fluid contents; sometimes small areas of redden-

ing will be detected, the rest of the gut being normal. The serous surface is commonly injected, as is practically the whole serous surface within the abdominal cavity.

The liver may be paler than usual; it may be more or less mottled, or, as is more frequently the case, present no macroscopic departure from normal. The gall bladder may be normal, or it may contain pale, thin, straw-coloured bile.*

The kidneys and adrenal bodies are usually acutely congested, especially the latter. The heart is filled with fluid blood. The lungs and spleen are, as a rule, normal. The retroperitoneal lymph glands are red and enlarged. When peritoneal involvement is not present in these cases the great serous cavities contain no excess of fluid.

Cultures from the site of inoculation always reveal the vibrios in large number. Those from the blood and internal organs may be sterile, they may all contain vibrios in small number, or colonies may appear on some tubes while others are sterile. It is rare that colonies in large number appear on any but the tubes from the site of inoculation. When peritonitis occurs as an accompaniment of subcutaneous inoculation vibrios are present in the peritonæum in large number.

When death does not occur for from fifty to sixty hours after inoculation, as is sometimes the case when small doses have been used, the blood and internal organs are found to be sterile, though living vibrios may in some instances be recovered from the site of inoculation.

When the subcutaneous dose has not been sufficiently large to cause death, a painful tumefaction almost always occurs about the

* Animals that survive inoculation, both subcutaneous and intra-peritoneal, for several days, and those in which death has occurred only after repeated inoculation with small doses of the living culture, or the toxine derived from old cultures, always present a conspicuous condition of the gall bladder and its contents. The gall bladder is distended, commonly very much so, with very pale, straw-coloured, watery bile, in which float numerous yellowish flakes of amorphous material. By culture tests the bile is found to be sterile. These animals are also markedly emaciated. A somewhat similar condition of the bile has been observed by Blachstein (*Johns Hopkins Hospital Bulletin*, July, 1891) in rabbits dead after chronic infection with bacillus coli communis. I have not, however, noticed the peculiar necroses of the liver that occurred in Blachstein's cases.

site of injection. This lasts for several days, growing gradually smaller and less tender from day to day. Finally the overlying skin sloughs away and leaves an open ulcer. This readily heals under a scab unless intercurrent infection occurs.

By Intraperitoneal Inoculation.—The fatal dose for guinea-pigs varies from 0.4 to 0.5 cubic centimetre of a normal suspension made from a twenty-four-hour-old agar culture.

When a dose not much above the certainly fatal amount is employed death occurs in from eighteen to twenty-two hours. It is at times a little later or a little earlier than this. When a much larger dose is used death may occur in as short a time as four or five hours.

The condition of the animal after inoculation is characterized by a gradually increasing condition of bodily depression that is accompanied by a conspicuous fall in the rectal temperature. The fall in temperature begins usually in about an hour to an hour and a half after the inoculation and continues steadily until death, by which time the temperature may have fallen to as low as 27° to 28° C. In one instance I saw the rectal temperature as low as 26.2° C. fifteen minutes before death. Sometimes there is a temporary rise in temperature for a short time after inoculation; this is, however, quickly followed by a gradual decline.

The general appearance of the animal is characteristic. It at first sits quietly in its cage, its hair becomes roughened, then its extremities are periodically extended, it settles on its belly on the bottom of the cage, often with its hind legs fully extended; from time to time there is convulsive jerking, and more or less constant trembling is seen for an hour or more preceding death. Respiration is accelerated, the nose and feet are cool, the abdomen is very sensitive to the touch and is moderately tense. When found dead the animal is usually flat upon its belly with legs stretched out behind it and its nose in the corner of the cage.

At autopsy the most conspicuous lesions are those of the peritonæum. It usually contains a few cubic centimetres of clear tenacious fluid. Over the surface of the liver, spleen, omentum, and intestines are scattered small patches of fibrino-purulent material. On

microscopic examination these are seen to consist very largely of leucocytes, with an occasional endothelial cell. If the dose has been just sufficient to cause death, numerous stained granules are also present; these are seen lying both free and within the bodies of leucocytes. Typical vibrios are not detected, as a rule. If the dose has been very large, microscopic examination reveals the presence of vibrios in large number. Often both typical vibrios and irregular stained granules are to be seen together. Occasionally more or less degenerated, though still recognisable, vibrios are found within the bodies of leucocytes. The pleural cavity may also contain a small amount of fluid in which leucocytes and stained granules are to be seen by microscopic examination.

The surface of the liver is irregularly covered with a thin deposit of fibrino-purulent material; otherwise the organ appears normal.

The serous surfaces of the intestines, as well as the serous surfaces generally within the abdominal cavity, especially the omentum, are more or less injected. Sometimes this is quite marked, while again it may be very limited in degree. The mucous membrane of the intestines is not usually inflamed.

The heart is relaxed and filled with fluid blood. The lungs are normal. Beyond a varying degree of congestion the other organs show no macroscopic changes.

By both microscopic and culture methods the peritonæum and internal organs are usually found to be sterile, or practically so, when the dose injected into the animal does not exceed the minimum lethal dose. If the dose is large, the peritonæum contains living vibrios in enormous numbers. Cultures from the blood and internal organs may reveal the presence of vibrios, though they usually occur in relatively small number in the blood; in a few cases I have found the blood to be sterile. Should death be postponed for forty-five to fifty hours, the peritoneal cavity, blood, and internal organs are sterile.

Inoculation of Mice.—The studies upon mice have not been as extensive as those upon pigeons and guinea-pigs, but they have been sufficient to justify the following statements: The results of subcutaneous inoculation of mice with this organism depend upon the

size of the dose employed. If very small doses be employed it is possible to cause death with all the evidences of a general infection, whereas if the dose be very large death occurs within a few hours and is manifestly the result of the toxic rather than the infective action of the material used. For example: Of two mice into the subcutaneous tissues of which (at the root of the tail) 0.05 cubic centimetre of a twenty-four-hour-old bouillon culture was injected, one died in twenty-four hours and a half, and the other recovered with a slough of the skin over the point of inoculation. At autopsy upon the one that died no macroscopic lesions were noticed beyond a slight œdema surrounding the reddened tissues at the site of injection. Cultures from this animal revealed the presence of vibrios at the site of inoculation in large number, and in the blood, liver, spleen, and kidneys in smaller numbers. If a much larger dose be employed, such, for instance, as 0.5 cubic centimetre of a similar culture, death occurs very rapidly, evidently the result of acute intoxication. Of two mice into the subcutaneous tissues of each of which such a dose was injected, one died in four hours and fifteen minutes and the other in four hours and twenty-five minutes after the treatment. At autopsy on these animals little beyond a red and swollen condition of the subcutaneous lymphatic glands was observed. Cultures revealed the presence of vibrios at the site of inoculation and in the internal organs.

The course of the disease and the post-mortem conditions found in all animals leave little room for doubt that death results more from the toxic activities of the organism than from its powers of general infection, though it nevertheless possesses this latter property. Notwithstanding this I have not been able to produce death of susceptible animals by the injection of dead cultures, killed by either chloroform or heat, in doses of several fold that of the living cultures, or by moderately large doses (1 cubic centimetre) of the filtrate from twenty-four-hour to thirty-six-hour bouillon cultures that were filtered through porcelain. Old fluid cultures—thirty-five to forty-five days of age—even when killed by chloroform, are highly toxic, and with them the characteristic lesions and death may readily be produced.

Immunity.—Scarcity of time at my disposal has prevented me from making a complete or systematic study of Vibrio Schuylkiliensis from this standpoint. Incidentally, however, a sufficient number of observations have been made to indicate a striking analogy between its protecting properties and those exhibited by the other suspicious spirilla embraced in this group.

Briefly stated, the results of these observations are as follows: In both pigeons and guinea-pigs immunity from relatively large doses of this organism may readily be induced either through the induction of tolerance to the poison contained in old dead cultures (destroyed by chloroform) or by the repeated employment of non-fatal doses of living cultures.

A single inoculation of either a pigeon or a guinea-pig with a non-fatal dose of living culture confers a condition of immunity that enables the animal to withstand a subsequent inoculation of from two to three times the minimal fatal dose. This immunity lasts for at least twelve days, and often longer. The phenomenon is especially marked in pigeons. An interesting process is often observed in the course of efforts to immunize guinea-pigs by intraperitoneal inoculation. If a guinea-pig be inoculated by this method with a dose somewhat below the minimal fatal dose, and within a few days be reinoculated in the same way with a similar dose, death sometimes follows the second inoculation, though the first may have been borne with little apparent inconvenience. This accords with an occasional observation of Pfeiffer[1] on the reaction of immune guinea-pigs to intraperitoneal inoculation with Vibrio choleræ Asiaticæ. Pfeiffer explains the phenomenon—that is, the death of the protected animal—by assuming that the amount of toxin liberated through the peculiar disintegration of the vibrios, referred to above, is greater than the amount against which the animals had been protected.

This observation suggested the possibility of there being a similar disintegration of the living vibrios in the peritoneal cavity of guinea-pigs rendered immune from Vibrio Schuylkiliensis. With this in view, two guinea-pigs that were partially immune, having withstood two previous intraperitoneal inoculations, were selected. Into the peri-

tonæum of each a little more than the fatal dose of a suspension of a fresh culture was injected. In ten minutes portions of the peritoneal exudate, obtained by means of capillary pipettes, were withdrawn and subjected to microscopic examination. A great variety of objects were seen, conspicuous among them being vibrios in all stages of involution. They were swollen, distorted, stained feebly, and were manifestly undergoing degeneration. Granules that took on the staining were also to be seen. After fifteen minutes the only objects found in the peritonæum were numerous granular masses, with an occasional body that might have been a vibrio, though it was impossible to state definitely. Numerous leucocytes were present. Occasionally stained granules were seen within the body of a leucocyte.

At fifteen minutes one of the guinea-pigs was killed. Cultures showed its peritoneal cavity to be sterile.

At thirty minutes the other was killed and its peritonæum was also found to be sterile.

If a much larger dose was employed the phenomenon was not observed, as the animals were evidently not immunized to a sufficiently high degree to withstand excessive amounts.

This germicidal activity of the peritonæum of immune guinea-pigs is, as we now know, not peculiar to animals immune from Asiatic cholera, but may also be observed in animals immune from other forms of infection as well; for instance, in animals immune from infection and intoxication by the bacillus typhi abdominalis and by the bacillus coli communis.

It would have been interesting to have determined the relation of these animals to intraperitoneal inoculation with the cholera spirillum, but as reliable cholera cultures were not at my disposal I was obliged to omit the experiment.

The tests that have been made with the serum from both pigeons and guinea-pigs that have been rendered immune from moderately large doses of Vibrio Schuylkiliensis show that it contains a substance that is capable of protecting susceptible animals against the pathogenic effects of otherwise fatal doses. Thus, for instance, in one experiment, 1 cubic centimetre of serum from a pigeon immune from

three times the fatal dose of living culture, when injected into the peritoneal cavity of each of two guinea-pigs, protected one of them against the fatal and the other against double the fatal dose of living culture injected at the same time as the serum. In another experiment of the same nature two out of three guinea-pigs were protected against double the fatal intraperitoneal dose of living culture by the simultaneous injection of 1 cubic centimetre of serum from a pigeon immune from three times the fatal dose of culture. The third animal of this series did not die until the end of the second day, showing a retardation of at least twenty-four hours in the appearance of the fatal result.

In another experiment a suspension was made by finely dividing a fresh culture on agar-agar in the serum from a pigeon immune from four times the fatal dose in such proportions that 0.5 cubic centimetre of the serum contained double the amount of culture necessary to produce fatal results. Into the pectoral muscle of each of two susceptible pigeons 0.5 cubic centimetre of this suspension was injected in less than five minutes from the time it was made. Neither pigeon showed any ill effects of the treatment.

In still another experiment it was found that when the serum from pigeons immune from at least three times the fatal dose was injected in the amount of 0.5 cubic centimetre into the pectoral muscles of susceptible pigeons, and this was followed within an hour by an injection into the muscle of the opposite side of double the fatal dose of a fresh normal suspension of the organism, no protective power was exhibited.

A similar property was seen to be possessed by the serum of guinea-pigs immune from double the fatal intraperitoneal dose of living vibrios. Thus, for example, the serum from such an immune guinea-pig when injected into the peritoneal cavity of each of two susceptible guinea-pigs in the dose of 1 cubic centimetre protected one of them against the simultaneous intraperitoneal injection of a little more than the fatal dose of living culture, and postponed the death of the other for three days. In all cases the control animals inoculated with the same dose of living culture, but which did not re-

ceive the serum, died after the proper interval with the characteristic lesions. On other controls that received only the serum no effect was produced.

I failed to observe with this serum the peculiar microscopic phenomenon, described by Pfeiffer and Vagedes,[3] that is seen to occur when the cholera spirillum is studied in a hanging drop made with the serum of very highly immunized animals. The failure to observe this reaction is probably due to the fact that my animals had been brought to only a very low degree of immunity.

Because of the many points of resemblance between the organism here described and Vibrio Metchnikovi, an effort was made to determine if pigeons rendered immune from Vibrio Schuylkiliensis were also immune from Vibrio Metchnikovi, and *vice versa*. The results show that such a reciprocal immunity occurs and persists for at least a week or ten days. It must be remembered, however, that this does not of necessity signify identity, for it will be borne in mind that Issaeff and Ivanoff,[8] in their studies upon the immunity induced by Vibrio Ivanovi, found that animals protected against it were also protected against Vibrio choleræ Asiaticæ. Nevertheless, the points of resemblance between Vibrio Schuylkiliensis and Vibrio Metchnikovi are so conspicuous and the points of difference so slight, being only quantitative, that I am constrained to consider them as very closely allied, if not identical. I can not at this time, however, consider them identical, for the reason given—namely, that it has thus far been impossible to increase the virulence of Vibrio Schuylkiliensis for pigeons to anything approaching the degree possessed by Vibrio Metchnikovi. In experiments with the latter organism I have found that very much attenuated cultures may quickly be brought to such a degree of virulence, by successive passage through pigeons, that the characteristic pathogenic powers will be exhibited when very small doses are used, such, for instance, as a minute particle on the point of a straight needle; this when rubbed into a superficial wound of the pectoral muscle of a pigeon causes death in about twelve hours with general invasion of the body by the organisms. (Pfeiffer makes a similar statement concerning his experience with this organism.)

Whether the organism under consideration is or is not a modification of Vibrio Metchnikovi, or is or is not identical with either of the hitherto described species, is of but little moment for the present. The important point to be borne in mind is, that as obtained from the waters in which it was found it was so anomalous in its pathogenic peculiarities as to make it impossible to do more than include it in the general group of suspicious vibrios. Its comparatively low infective properties for pigeons, its characteristic reaction by intraperitoneal inoculation of guinea-pigs, and the majority of its cultural and morphological peculiarities might readily lead one to mistake it for an organism of much more important significance, especially if surrounding circumstances, such as the presence of suspicious intestinal troubles, would seem to justify such an opinion.

In the light of this observation I think we can safely say that there exist pathogenic spirilla, sufficiently similar to the cholera spirillum to justify their being classified as suspicious, which manifestly have nothing to do with the disease cholera. I can not avoid the conclusion that the discovery of this organism in the water of a locality absolutely free from suspicion, in so far as Asiatic cholera is concerned, serves as an additional refutation of the opinion that the cholera spirillum and the majority of the forms that simulate it are but variations of one and the same species. There may be a striking family likeness, but one produces cholera while the others do not; a distinction that, in the present state of our knowledge, can not be ignored, no matter what may be the outcome of subsequent studies of the causes underlying variability of species.

So far as I am aware, this is the first suspicious pathogenic spirillum that has been detected in the surface waters of this country. This is probably not due to their rarity or to their being peculiar to the waters of particular localities, but rather to the fact that few systematic efforts have been made to discover them.

If they are generally present it is important for us to know it, for in the event of an outbreak of cholera or of suspicious intestinal troubles of other nature they would undoubtedly prove the cause

of endless confusion and false interpretations were their presence unsuspected.

It is with the hope of awakening an interest in the subject among American bacteriologists and of obtaining general data on this important subject that this contribution is made.

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3. Pfeiffer and Vagedes.—*Centralbl. f. Bakteriol. u. Parasitenk.*, Bd. xix, p. 385.
4. Dunbar.—*Arbeit. a. d. kais. Gesundheitsamte*, Bd. ix, p. 379.
5. Koch.—*Zeitschr. f. Hyg. u. Infektionskrankh.*, Bd. xiv, p. 319.
6. Ivanoff.—Ibid., Bd. xv, p. 434.
7. Pfeiffer.—Ibid., Bd. vii, p. 347.
8. Issaeff and Ivanoff.—Ibid., Bd. xvii, p. 117.

Description of Plates.

All of the figures are photomicrographs of the Vibrio Schuylkiliensis. For Photographs 1 and 2 I am indebted to Dr. Charles L. Leonard, of the University of Pennsylvania.

Plate XVI.

Fig. 1.—Vibrio Schuylkiliensis. Normal forms as seen in fresh cultures on gelatin. × 1,000 diameters.

Fig. 2.—Involution forms. From potato culture forty-eight hours old. × 1,000 diameters.

Fig. 3.—Colonies in gelatin plate after twenty-four hours' growth at 18° to 20° C. × 100 diameters.

Fig. 4.—Colonies in gelatin plate after forty-eight hours' growth at 18° to 20° C. × 100 diameters.

Plate XVII.

Fig. 5.—Colony in gelatin after about fifty hours' growth at 18° to 20° C. × 100 diameters.

Fig. 6.—Colony in gelatin after about seventy hours at 18° to 20° C. × 100 diameters.

Fig. 7.—Colony in gelatin after about seventy hours at 18° to 20° C. × 100 diameters.

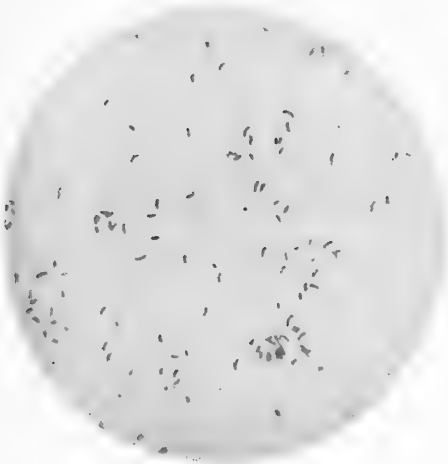
Fig. 8.—Colony in gelatin after about seventy hours at 18° to 20° C. × 100 diameters.

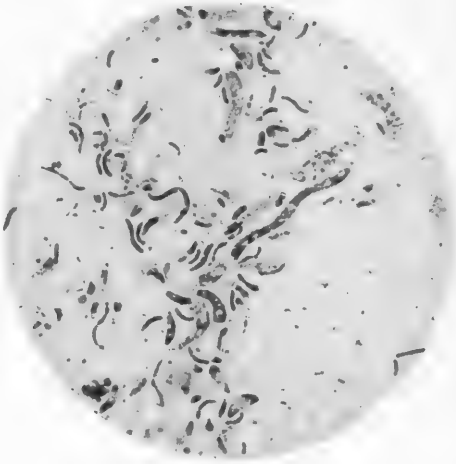
PLATE XVIII.

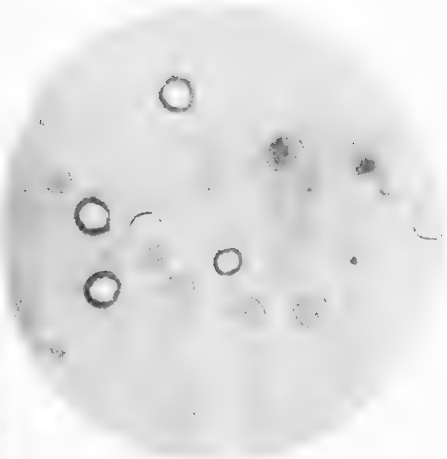
Fig. 9.—Colony in gelatin after three days and a half. At two days to two days and a half this colony showed distinct concentric markings. Temperature, 18° C. × 100 diameters.

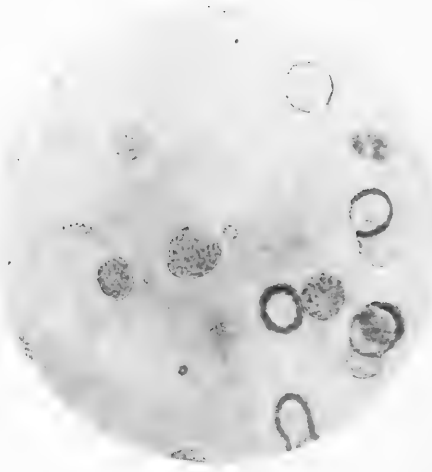
Fig. 10.—Edge of single colony in gelatin after two days and a half at 18° C. × 100 diameters.

Fig. 11.—Stab cultures in gelatin at 18° to 20° C.: *a*, after four days; *b*, after seven days; *c*, after seventeen to eighteen days. Approximately natural size.









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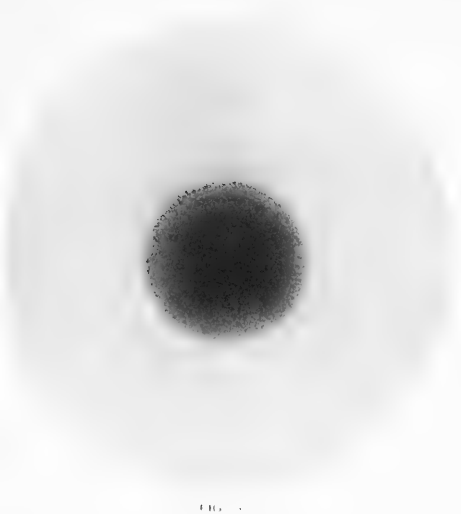

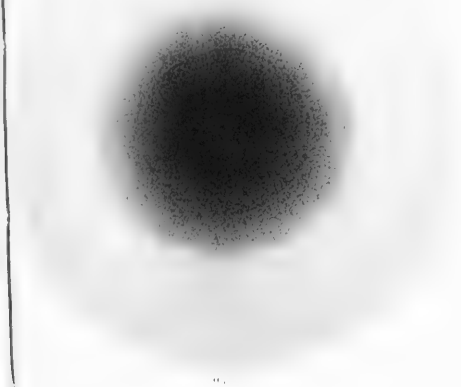

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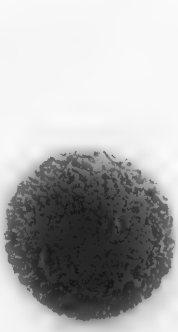

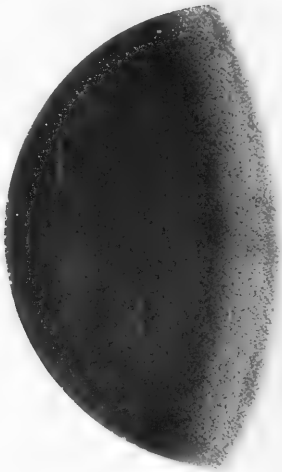

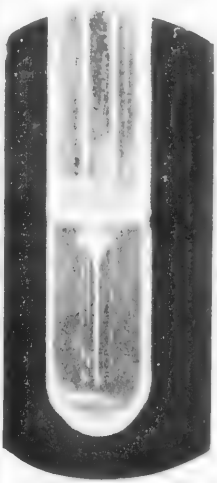

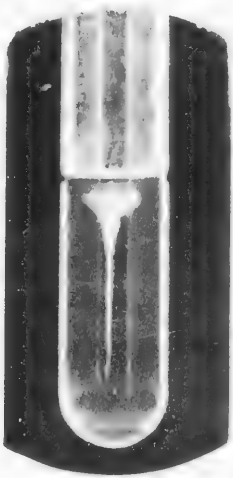

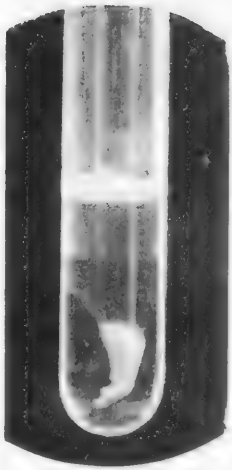

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THE INFLUENCE OF ACUTE ALCOHOLISM ON THE NORMAL VITAL RESISTANCE OF RABBITS TO INFECTION.

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During the past six months a portion of the time at my disposal has been occupied with experiments the results of which I propose to embody in this contribution.

These studies were made in the Laboratory of Hygiene of the University of Pennsylvania under the auspices of the Committee of Fifty to Investigate the Alcohol Question, who generously placed at my disposal the funds necessary to defray the expenses of the work.

In the distribution of the several subdivisions of the general question relating to the influence of alcohol upon the physical and moral well-being of those who use it as a beverage, the committee has seen proper to assign to me for experimental solution one of the most interesting and important phases of the subject—viz., that concerning the influence of alcoholism, acute and chronic, upon the normal vital resistance of animals to various forms of infection.

In undertaking the work a general scheme of procedure was drawn up that seemed to meet most of the requirements of the problem, but as I now review it I find that it has been possible, in the time at my disposal, to cover only a limited portion of the ground included in this scheme.

The lines along which it was proposed to conduct these investigations are as follows:

1. Determine if the normal vital resistance of animals to infection by the common pathogenic bacteria is demonstrably influenced

by either acute or chronic alcoholism, induced through the use of known amounts of pure ethyl alcohol.

2. If any or no effect is observed, determine if the same holds good for animals under the influence of the commoner alcoholic beverages, as beer, wine, whisky, cordials, etc.

3. Determine if through either acute or chronic alcoholism the germicidal properties of the serum of the blood of animals is materially altered. If so, determine, if possible, the nature of this alteration.

Thus far the work has borne upon the question concerning the influence of acute alcoholism upon the resistance of rabbits to infection by the pyogenic cocci—i. e., the streptococcus pyogenes (erysipelatos) and the staphylococcus pyogenes aureus—and by the bacillus coli communis, and the results are in many ways sufficiently instructive to warrant their report at this time.

A difficulty that arose at the outset was in procuring cultures of pathogenic organisms of suitably diminished virulence without their being at the same time totally devoid of this property. As experience has taught us to expect certain deviations from the usual course of infection when normal animals are inoculated with attenuated cultures, such, for instance, as prolongation of the period of incubation and modification of the pathological lesions, the problem under consideration seemed to be in part most easy of solution through a comparison of results obtained by the use of such cultures on groups of alcoholized and non-alcoholized animals. Should such modifications occur in the normal animals, as would be anticipated, and an infection not differing from that produced by fully virulent cultures occur in the alcoholized animals, the difference could reasonably be referred to a reduction of vital resistance brought about by the alcohol administered.

To meet the requirements it would perhaps have been best to have employed cultures of organisms that were diminished in virulence to definite degrees of attenuation, such, for instance, as the bacillus anthracis attenuated to the degree of virulence represented by its primary and secondary vaccines. As such material was not at my disposal, however, cultures of another sort were used—namely,

those that were of a low degree of virulence without this degree being definitely determined.

Again, cultures of organisms were employed the pathogenic powers of which are usually irregular and uncertain, while in still other tests an effort was made to detect a difference between alcoholized and non-alcoholized animals when inoculated with virulent material but in very small doses, hoping in this way to demonstrate, by a difference in the period of incubation, a difference of resistance in the animals composing the two groups.

The experiments were made with cultures of streptococcus pyogenes of a low degree of virulence, with normal bacillus coli communis, and with virulent staphylococcus pyogenes aureus, and, as the results to be reported show, with very diverse consequences.

Another difficulty was encountered in regulating the dose of alcohol. The intention was to have the animals daily in a state of intoxication, but it is not easy to decide just when this stage is reached, as the only certain indication of it is inco-ordination of muscular movement. It frequently occurred that when alcohol was given to this stage the direct effect of the drug was such as to gravely imperil the life of the animal, and in a certain number of instances the animals did not rally from even so few as one or two such administrations. If the dose were diminished, then one could often not be sure that the rabbit was intoxicated.

On the other hand, one encounters occasionally an individual on which alcoholism to intoxication has apparently no effect upon the general health, and may be prolonged over relatively long periods. One of my animals, for instance, received daily doses of alcohol ranging from 5 to 15 cubic centimetres for one hundred and fourteen days. Its weight at the beginning was 1,330 grammes, and at the end 1,350 grammes, while during the interval its weight had been as high as 1,520 grammes and as low as 1,090 grammes. It died under the administration of 15 grammes daily with macroscopic lesions of only the gastric mucous membrane. I believe that the studies upon the effects of smaller, non-intoxicating doses of alcohol will demonstrate the possibility of continuing the administration of the drug over prac-

tically an indefinite period, quite to the stage of chronic alcoholism, in a fair proportion of the animals treated in this way.

The individual susceptibility of different animals to the physiological action of alcohol differed in such a way as to require at the beginning a special dose for each individual. It is impossible to maintain throughout a constant relation between body weight and dose necessary to produce the desired effects. For instance, it can not be said if 5 cubic centimetres of alcohol produces intoxication in a rabbit of 1,000 grammes weight, that necessarily 10 cubic centimetres will have the like effect in the next rabbit of 2,000 grammes weight. The dose can only be determined by experiment.

It frequently occurred that, when the necessary dose had been determined, its continuous administration over several or more days resulted in very rapid and marked loss in body weight, an indication of the greatest importance, meaning, that if the alcohol be continued the animal is practically certain to die. Under these circumstances it becomes necessary to either diminish the dose or cease the administration entirely for a time, until the lost weight is partially or completely recovered. During the interval of rest, as it may be called, the animal is of course not daily intoxicated, and while the records of these experiments refer to animals "daily under the influence of alcohol to the stage of intoxication," it will be borne in mind that with many of these animals such periods of rest, varying in duration, were more or less frequently necessary.

As it was impossible to induce the rabbits to take alcohol voluntarily, either with the food or otherwise, it became necessary to administer it through a soft-rubber catheter passed down the œsophagus into the stomach, and though this was done as gently as possible, the irritation resulting from the repeated passage of the catheter, together with the depressing influence of the drug, was accompanied in a number of instances by intercurrent infections which, from their character and location, I think can be reasonably traced to insignificant wounds of the mucous surface of the œsophagus made when the alcohol was administered.

In only two instances was death positively the result of inspira-

tion of alcohol into the air passages at the time of operation. When such an accident occurs death is practically instantaneous.

The most common macroscopic effect of the direct action of the alcohol was erosion and inflammation of the mucous membrane of the stomach, a condition that was unfortunately present in quite a number of my animals, and which complicated matters in such a way as to vitiate considerably the results of the experiments.

When the necessary dose of alcohol was determined, it was always given mixed with an equal quantity of distilled water.

The dose necessary to positively produce intoxication ranged from 5 cubic centimetres to 15 cubic centimetres of pure ethyl alcohol, though the usual dose in rabbits of from 1,500 to 1,800 grammes weight was from 7.5 cubic centimetres to 10 cubic centimetres, doses which if taken by a man of one hundred and fifty pounds weight would be roughly equivalent to from two thirds to five sixths of a pint of absolute alcohol. Notwithstanding this apparently very large amount, animals have repeatedly been encountered on which from 10 to 12, and even 15 cubic centimetres had no visible influence, though the continuance of these doses in many instances was followed by death associated with grave lesions of the gastric mucous membrane.

While rabbits offer conditions in a way favourable to experiments of this character, they are so liable to intercurrent bacterial and protozoal infections as to lead to many irregularities when large numbers of them are used. In the course of this work a number of experiments have been seriously vitiated by reason of such complications. The general plan followed in these experiments was to employ groups of equal numbers of animals. All were inoculated in the same way, then to one group alcohol was given, while to the other no alcohol was administered. They were all then kept under the same conditions of food, etc. Each animal was weighed in the morning before it was fed. In one or two cases the number of " controls " was fewer than that of the alcohol group. Control animals receiving only alcohol were not introduced into each experiment, as the necessary data on this point could always be had by reference to the records of

preceding experiments in which the different effects of the drug on different animals was recorded.

Without going further into the particulars, it will suffice to tabulate the details and briefly summarize the results of each experiment as follows:

Experiment I.

Summary of the results of the intravenous inoculation of rabbits receiving daily doses of alcohol to the stage of intoxication, with bouillon cultures of streptococcus pyogenes obtained from a phlegmonous inflammation in man:

Of eleven rabbits inoculated intravenously with varying amounts of streptococcus pyogenes, six received daily doses of alcohol for different lengths of time. The remaining five of this group received no alcohol. In addition, two rabbits received only daily doses of alcohol. Of the six inoculated animals that received alcohol, all died after marked loss of weight and all exhibited lesions referable to the inoculation. One revealed a condition of the liver probably the result of the action of the alcohol.

Of the five inoculated animals to which no alcohol was given, one died, one was killed, and three recovered.

The one that died presented no lesions referable to the inoculation. Cultures revealed the presence of streptococci in the liver mixed with another organism, while the other internal organs were sterile.

The animal that was killed presented no lesions of importance, and its blood and internal organs were found by culture test to be sterile.

Each of the three remaining animals suffered from temporary erysipelas of the ear at the site of needle puncture. They recovered from this, and at the end of one hundred and twenty days were apparently in good condition, one having gained 330 grammes, the other 110 grammes, and the other 270 grammes in weight.

Of the two animals that were not inoculated, but which received alcohol daily, one died after one hundred and fourteen days with a condition of the gastric mucous membrane referable to the alcohol, the other after thirty-four days with no macroscopic lesions to account for death.

Experiment II.

Summary of the results of intravenous inoculation of rabbits, receiving intoxicating doses of alcohol daily, with suspensions of streptococcus pyogenes:

The eighteen animals employed in this experiment were divided into four groups and treated as follows:

Four received intoxicating doses of ethyl alcohol for five days and were then inoculated intravenously with 0.5 cubic centimetre of a suspension of streptococcus pyogenes.

Five received intoxicating doses of methyl alcohol for five days and were then similarly inoculated.

The alcohol was continued in both groups after inoculation until death.

Five received, in addition to the inoculation, daily doses of distilled water, injected into the stomach in the same manner as was the alcohol in the other animals, for fifteen days. After this alcohol was given to two of them—to one for two days, to the other for six days—while the remaining three got nothing from the fifteenth day.

Four were simply inoculated. One of this group received a single dose of alcohol.

The nine latter animals served as controls.

Results.—Of nine inoculated animals to which alcohol was given, seven died with suppurative lesions.

Of nine inoculated animals that received no alcohol until after the fifteenth day, when alcohol was given to two, five died, three were killed, and one was observed for fifty-two days. In only three of the nine were suppurative lesions detected at autopsy.

Of the seven alcohol animals that died, five presented lesions at autopsy that could be attributed to the action of the alcohol.

Experiment III.

Summary of the results of intravenous inoculation of rabbits receiving alcohol daily to the stage of intoxication, with suspensions of bacillus coli communis from human fæces:

Of five rabbits, each of which was inoculated with 1 cubic centimetre of a suspension in bouillon of bacillus coli communis, and all of which had

been receiving alcohol to the stage of intoxication daily for five days, four died in from eighteen to twenty hours and one in eighteen days after inoculation.

None presented lesions referable to the inoculation. One revealed a condition of the gastric mucous membrane that evidently resulted from the direct action of the alcohol. Bacillus coli communis was obtained in cultures from the internal organs of all.

Five rabbits, each of which had been receiving alcohol in doses ranging from 2.5 to 15 cubic centimetres daily for thirty-one days, were inoculated intravenously with the same material in the same amount that was employed in the preceding group.

One died within twenty-four hours after the inoculation.

Four died in from forty-three to fifty-five days after the inoculation.

Three presented at autopsy lesions referable to the inoculation—viz., the condition of the liver, gall bladder, and bile described by Blachstein.*

Two presented no lesions attributable to the inoculation.

Two showed a condition of the liver that may possibly have resulted from the prolonged action of the alcohol, while in the remaining three no results of the alcohol were evident.

Two control animals that were inoculated in the same manner with the same material, but which received no alcohol, were alive on the seventy-ninth day after inoculation. By the sixtieth day one had gained 230 grammes in weight, while the other, though apparently well, had lost 200 grammes in weight.

Experiment IV.

Summary of the results of inoculation of alcoholized animals with suspensions of bacillus coli communis:

Three animals received intravenous inoculation of 0.5 cubic centimetre of a suspension of bacillus coli. Immediately afterward the administration of alcohol was begun. One died during the night and one on the day following the inoculation. Bacillus coli was found by cultures in the internal organs of both. The third died eight days after inoculation with no characteristic lesions and no colon bacilli in the internal organs.

* Blachstein. Intravenous Inoculation of Rabbits with the Bacillus Coli Communis and the Bacillus Typhi Abdominalis. *Bulletin of the Johns Hopkins Hospital*, vol. ii, No. 14, July, 1891.

The mucous membrane of the stomach was eroded and there was a fibrino-purulent pericarditis and pleuritis, evidently the result of an intercurrent infection, probably induced by the repeated passage of the catheter along the œsophagus.

Three other rabbits that had been getting alcohol for four days were inoculated in the same manner as the preceding. Two died on the ninth day and one on the eighteenth day following inoculation.

In one, dead on the ninth day, were the characteristic lesions described by Blachstein. There was also present a condition of the stomach that was manifestly the result of the action of the alcohol.

Of the remaining two, in one death ensued from an intercurrent infection, while in the other death was the result of an intestinal intussusception. This latter animal presented erosion of the gastric mucous membrane that was evidently the result of the action of the alcohol.

In both animals that died on the ninth day colon bacilli were present in cultures from the internal organs, while in the one dead on the eighteenth day the blood and internal organs were sterile.

Three control animals were alive and apparently well on the forty-sixth day. One had gained 50 grammes, the other 280 grammes, while the third had lost 120 grammes in weight.

In short, of six alcoholized rabbits inoculated with colon bacilli, all died. One presented characteristic lesions at autopsy. Of three non-alcoholized, control animals, none died.

Experiment V.

Results of intravenous inoculation of rabbits receiving alcohol, with cultures of staphylococcus pyogenes aureus:

In this experiment the first inoculation was without effect, and the animals were inoculated a second time after an interval of seven days. Four animals were employed—viz., two alcoholized and two controls. The alcoholized animals died in fourteen and two days, respectively, after the inoculation. Both presented lesions referable to the inoculation and neither showed conditions due to the direct action of the alcohol. One control animal died in four days after the first inoculation, while the second died in six days after the second inoculation. To this latter animal alcohol was administered from the time of the second inoculation. Both presented lesions referable to the inoculation, and the one that got alcohol presented also conditions due to the direct action of the alcohol.

This experiment does not demonstrate any material difference between the alcoholized and the non-alcoholized animals as regards their susceptibility to this form of infection.

Experiment VI.

Subcutaneous inoculation of alcoholized animals with cultures of staphylococcus pyogenes aureus:

There were four animals in this group, two alcoholized and two controls. In all except one, an alcoholized rabbit, the subcutaneous inoculation was without effect up to the seventh day. On the seventh day the three remaining animals were inoculated again, but this time intravenously.

Results.—One alcoholized animal died in three days after the subcutaneous inoculation. Death was due to pseudo-tuberculosis of the lungs. There was only a trifling area of suppuration at the seat of inoculation. The other alcoholized rabbit died four days after the intravenous inoculation and presented the usual condition of widespread miliary-abscess formation. Both control animals died after the intravenous inoculation—the one in seven days, the other in twenty-four hours. The former presented lesions characteristic of the inoculation, while the latter presented no macroscopic lesions at all at autopsy. The former control animal had been given alcohol since the date of its second intravenous inoculation. At autopsy it presented lesions referable to the direct action of the alcohol.

Experiment VII.

Intraperitoneal inoculation of alcoholized animals with cultures of staphylococcus pyogenes aureus:

Four animals were employed; two received alcohol and two served as controls.

The animals that exhibited no effects of the intraperitoneal inoculation at the end of a week were reinoculated with the same material, but this time the inoculation was intravenous.

One alcoholized animal died the day following the intraperitoneal inoculation, the other two days after the intravenous inoculation.

In neither were lesions detected that could account for death, or be positively referred to either the inoculation or the alcohol.

Both control animals recovered from the intraperitoneal inoculation and at the end of a week were inoculated intravenously.

One died two days after this latter inoculation and presented neither mycotic nor alcoholic lesions, death being due to rupture of the gravid uterus, while the other died nine days after intravenous inoculation with abscesses in myocardium and kidneys.

Experiment VIII.

Intravenous inoculation of alcoholized rabbits with bouillon cultures of staphylococcus pyogenes aureus:

Of the six inoculated animals to which alcohol was given, three died in less than twenty-four hours and three in from forty-eight to seventy-two hours after inoculation. In three, lesions were present that could positively be referred to the inoculation, and in one the condition was doubtful. In two no such lesions could be found.

In five of the six animals a condition of the mucous membrane of the stomach was found that was evidently the result of the direct action of the alcohol.

Of the six inoculated animals that did not get alcohol three died in less than twenty-four hours, two after five and twelve days, respectively, while the remaining animal was apparently well at the end of forty-three days.

Three of these animals presented lesions that resulted from the inoculation, in one the lesion was doubtful, in one no lesions were detected, and the sixth is still alive.

This experiment demonstrates a slight, though not very important or striking, difference between the course of infection in the two groups.

Experiment IX.

Subcutaneous inoculation of alcoholized rabbits with cultures of staphylococcus pyogenes aureus:

Of the six inoculated animals to which alcohol was given, one died during the night following the inoculation; the remaining five died in from five to fifteen days later.

Two of these animals presented no lesions referable to the inoculation, one presented an extensive local lesion, and three presented a lesion of the pleuræ and pericardium of doubtful significance. This condi-

tion was identical in the three animals and consisted of a fibrino-purulent inflammation of practically all the serous surfaces within the thoracic cavity. Cultures from this lesion revealed the presence of a staphylococcus that grew feebly, producing but a very faint, barely perceptible, cream colour, and in general gave the impression that it might be an attenuated or enfeebled culture of staphylococcus aureus. It was not studied in detail.

The animals in which this lesion was found died in five, fifteen, and fifteen days, respectively.

The three control animals used in this experiment manifested no ill effects of the treatment. They were alive and well at the end of forty-six days after inoculation.

In none of the six animals that received alcohol was there any macroscopic evidence of the action of the alcohol on the tissues.

Experiment X.

Intravenous inoculation of alcoholized rabbits with staphylococcus pyogenes aureus attenuated (?) by exposure to chloroform:

Four animals were used in this experiment.

Of the two inoculated animals to which alcohol was given one died on the fifth, the other on the second day following the inoculation. One presented a lesion of the kidney positively referable to the inoculation, the other a condition of the peritonæum that probably, though not certainly, resulted from the treatment to which the animal had been subjected.

Both of these animals presented extensive erosions of the gastric mucous membrane that was manifestly the result of the direct action of the alcohol.

Neither of the two control animals evinced any ill effects from the inoculation; both gained in weight, and at the end of twenty-eight days were eliminated from the experiment as not likely to manifest evidences of infection.

Conclusions.

The deductions that may be drawn from the results of these experiments are as follows:

That the normal vital resistance of rabbits to infection by streptococcus pyogenes (erysipelatos) is markedly diminished through the

influence of alcohol when given daily to the stage of acute intoxication. That a similar, though by no means so conspicuous, diminution of resistance to infection and intoxication by the bacillus coli communis also occurs in rabbits subjected to the same influences.

And that, while in alcoholized rabbits inoculated in various ways with staphylococcus pyogenes aureus, individual instances of lowered resistance are observed, still it is impossible to say from these experiments that in general a marked difference is noticed between alcoholized and non-alcoholized animals as regards infection by this particular organism.

It is interesting to note that the results of inoculation of alcoholized rabbits with the erysipelas coccus correspond in a way with clinical observations on human beings addicted to the excessive use of alcohol when infected by this organism.

In the course of the work an effort was made to determine if, through the oxidation of alcohol in the tissues to acids of the corresponding chemical group, the increase of susceptibility could be referred to a diminution in the alkalinity of the blood as a result of the presence of such acids. The number of experiments thus far made on this point is too small to justify dogmatic statements, but from what we have gathered there is but little evidence in support of this view.

Throughout these experiments, with few exceptions, it will be seen that the alcoholized animals not only showed the effects of the inoculations earlier than did the non-alcoholized rabbits, but in the case of the streptococcus inoculations the lesions produced (formation of miliary abscesses) were much more pronounced than are those that usually follow inoculation with this organism.

With regard to the predisposing influence of the alcohol, one is constrained to believe that it is in most cases the result of structural alterations consequent upon its direct action on the tissues, though in a number of the animals no such alteration could be made out by macroscopic examination. I am inclined, however, to the belief, in the light of the work of Berkley and of Friedenwald, done under the direction of Prof. Welch, in the Pathological Laboratory of the

Johns Hopkins University, that a closer study of the tissues of these animals would have revealed in all of them structural changes of such a nature as to indicate disturbances of important vital functions of sufficient gravity to fully account for the loss of normal resistance.

The conspicuous influence of the alcohol on the gastric mucous membrane in many of these animals, with the consequent disturbance of nutrition, is undoubtedly the explanation of the marked loss in body weight that was observed in many of the animals employed in these experiments. In this light the susceptibility induced by alcohol to excess is somewhat analogous to that induced by starvation, where we see the resistance of animals to particular forms of infection very markedly diminished.

TABULATED DETAILS OF FOREGOING EXPERIMENTS.

Experiment I.

Table showing the Results of Intravenous Inoculations of Rabbits that were receiving Daily Doses of Ethyl Alcohol to the Stage of Intoxication with Bouillon Cultures of the Streptococcus Pyogenes, obtained from a Phlegmonous Inflammation of the Foot of a Man.

Rabbits.	No. of days observed.	Total amount of alcohol received.	Daily dose of alcohol.	Interval between the beginning of alcohol and time of inoculation.	Weight in grammes.		Lesions referable to inoculations. + = present. O = absent.	Lesions referable to the alcohol. + = present. O = absent.	Results and remarks.
					Beginning.	End.			
1	41	250 c. c.	5 to 10 c. c.	36 days. Was then inoculated with 1 c. c. of bouillon culture of streptococcus intravenously.	1,190	910	+ General infection.	+ (?) (Liver.)	Died six days after inoculation. Liver very yellow and friable; contained large numbers of streptococci; many of the capillaries quite filled with them. Streptococci also present in pure culture in blood, kidney, and bone marrow. No abscesses detected.
2	40	240 c. c.	5 to 7·5 c. c.	35 days. Inoculation same as that of No. 1.	1,210	880	+ Miliary abscesses in muscles.	O	Died on sixth day after inoculation. Numerous miliary abscesses in muscles along the spine. No abscesses in myocardium or kidneys. *Cultures.*—Streptococci in pure culture in muscle abscesses, blood, liver, kidney, and spleen.
3	49	297·5 c. c.	5 to 7·5 c. c.	45 days. Intravenous inoculation of bouillon culture of streptococcus 24 hours old.	1,280	960	+ Miliary abscesses in muscles.	O	Died on fifth day after inoculation. Miliary abscesses throughout the voluntary musculature. Abscesses in myocardium. Pale areas, but no abscesses in kidneys. Spleen dark and congested. *Cultures.*—Streptococci in pure culture and in large numbers in muscle abscesses, blood, liver, spleen, and kidney.
4	47	282·5 c. c.	5 to 7·5 c. c.	45 days. Inoculation same as that of No. 3.	1,200	1,040	+ Abscesses (?) in myocardium. General infection.	O	Died on third day after inoculation. No abscesses in muscles. Several pale points in myocardium; these contained streptococci in large numbers on microscopic examination. *Cultures.*—Streptococci in pure culture from pale points in heart, from blood, liver, spleen, kidney, and peritoneal cavity.

Rabbits.	No. of days observed.	Total amount of alcohol received.	Daily dose of alcohol.	Interval between the beginning of alcohol and time of inoculation.	WEIGHT IN GRAMMES. Beginning.	WEIGHT IN GRAMMES. End.	Lesions referable to inoculations. +=present. O=absent	Lesions referable to the alcohol +=present. O=absent.	Results and remarks.
5	16	145 c. c.	7·5 to 10 c. c.	Inoculated 3 times. Intervals: First, 2 days; second, 4 days; third, 7 days. Last inoculation (fatal) was 0·8 c. c. of a bouillon culture of streptococcus intravenously.	1,740	1,290	+ Miliary abscesses in muscles.	O	Animal had two subcutaneous inoculations, first two days and second four days after beginning of alcohol. Seven days after beginning of alcohol it received an intravenous injection of 0·8 c. c. of a bouillon culture of streptococcus pyogenes. Died nine days after last inoculation. Sites of subcutaneous inoculations sterile. As result of intravenous inoculation the voluntary muscles were everywhere studded with miliary abscesses. Similar abscesses in diaphragm; none in internal organs. *Cultures* from abscesses in muscles and from blood show streptococci in pure culture.
6	3	30 c. c.	10 c. c.	Both given at same time. Same inoculations as those for No. 5.	1,910	1,740	+ Miliary abscesses in muscles.	O	Died in afternoon of second day after inoculation. Miliary abscesses throughout voluntary muscles, especially those of thorax. Spleen very large, almost black in colour; measured 6·5 cm. long, 2 cm. wide, and 1·5 cm. thick. Numerous abscesses in myocardium. Cultures and cover slips revealed streptococci in muscle abscesses, liver, spleen, blood, and kidney.
				Control Animals, received Alcohol, not inoculated.					
A	114	845 c. c.	5 to 15 c. c.	Not inoculated.	1,330	1,350	.....	+ Mucous membrane of stomach.	The daily dose of 15 c. c. was begun eight days before death, after the animal had been getting 10 c. c. daily for the thirty-seven preceding days. *Autopsy* revealed macroscopic lesions only in the stomach. The walls were thickened and œdematous, in some places thin. There was widespread erosion and necrosis of the mucous membrane.
B	34	147·5 c. c.	5 to 10 c. c.	Not inoculated.	1,030	600	.....	O	The animal was markedly susceptible to alcohol. At autopsy, no macroscopic lesions to account for death were found.

Control Animals inoculated, but received no Alcohol.

C Control for No. 1.	120	No alcohol.	.....	No alcohol. Inoculated at same time as No. 1, and in same way.	.....	.....	+ Erysipelas of ear about site of operation.	.....	In three weeks after inoculation this animal was well, having had a local erysipelas of ear at point of puncture of needle. After four months it had gained 330 grammes in weight, and was not observed further.
D Control for No. 2 and No. 6.	120	Do.	.....	No alcohol. Inoculated at same time and in same way as No. 2.	.....	.....	+ Temporary erysipelas of ear at site of operation.	.....	After about three weeks the animal was well of its local erysipelas in ear. It had recovered its lost weight, and was apparently in good condition. At end of four months it had gained 110 grammes in weight. Observation discontinued.
E Control for No. 3.	120	Do.	.....	No alcohol. Inoculated at same time and in same way as No. 3.	.....	.....	+ Temporary erysipelas of ear.	.....	Temporary redness and swelling of ear at site of operation. At end of four months had gained 270 grammes. Observation discontinued.
F Control for No. 4.	16	Do.	.....	No alcohol. Inoculated at same time and in same way as No. 4.	1,220	800	+	.....	Died after sixteen days. For some time had had profuse watery discharge from nostrils and mouth. This began on fourth day after inoculation. No lesions of importance found at autopsy. *Cultures.*—Blood: pure culture of streptococci. Liver: streptococci, with a large micrococcus. Lung, kidney, and spleen sterile.
G Control for No. 5.	15	Do.	.....	No alcohol. Inoculated at same times and in same way as No. 5.	1,410	1,590	+ Circumscribed pus formations at sites of subcutaneous inoculations.	.....	Animal killed on fifteenth day—*i. e.*, nine days after its last (intravenous) inoculation. No miliary abscesses in muscles and no lesions of internal organs found at autopsy. Circumscribed pus formations in stages of healing at both sites of subcutaneous inoculation. *Cultures* from sites of subcutaneous inoculation and internal organs showed them to be sterile.

Experiment II.

Table showing the Results of Intravenous Inoculation of Rabbits that were receiving Daily Doses of Alcohol to the Stage of Intoxication, with Suspensions of Streptococcus Pyogenes.

Ethyl Alcohol.

Rabbits.	No. of days observed.	Total amount of alcohol received.	Daily dose of alcohol.	Interval between the beginning of alcohol and time of inoculation.	Weight in Grammes.		Lesions referable to inoculations. +=present. O=absent.	Lesions referable to the alcohol. +=present. O=absent.	Results and remarks.
					Beginning.	End.			
7	13	142·5 c. c.	7·5 to 15 c. c.	5 days. Intravenous injection of 0·5 c. c. of a suspension of streptococcus pyogenes.	2,010	1,650	O	O	Died eight days after inoculation. *Autopsy.*—No lesions anywhere to be detected. No streptococci in cultures. Cultures all contain short oval bacillus. Evidently an intercurrent infection.
8	12	142·5 c. c.	7·5 to 15 c. c.	Do.	1,600	1,350	+ Muscle abscesses.	+ (Stomach.)	Died seven days after inoculation. Abscesses in muscles of both thighs; none in heart or kidneys. Mucous membrane of stomach very much eroded. *Cultures.*—Streptococci in large numbers and in pure culture from blood, liver, kidney, and muscle abscess.
9	10	90 c. c.	7·5 to 10 c. c.	Do.	1,020	940	+ Streptococci in blood.	O	Died fourth day after inoculation. *Autopsy.*—No abscesses anywhere in voluntary muscles. Several diffuse pale areas in heart; numerous necrotic areas in liver. *Cultures* demonstrate a mixed infection; those from blood and kidney contain streptococci, those from the other organs contain a short oval bacillus.
10	12	132·5 c. c.	7·5 to 15 c. c.	Do.	1,860	1,530	O	+ (Stomach.)	Died seven days after inoculation. *Autopsy.*—Muscle abscesses not detected. Liver contained coccidia nodules. Kidneys presented several small cortical hæmorrhages. Mucous membrane of stomach inflamed and eroded. *Cultures.*—No streptococci in cultures from blood, liver, spleen, kidneys, or peritoneal cavity. In all these tubes was a short oval bacillus.
11	.....	.....	.....	.....	.....	.....	.....	.....	Died on fourth day as result of alcohol. Had not been inoculated.

Animals receiving Commercial (Pure) Methyl Alcohol.

31	12	11	109 c. c.	3 to 15 c. c.	5 days. Inoculation same as in preceding animals of this experiment.	1,520	1,260	+ Streptococcus septicæmia.	+ (Stomach.)	Died six days after inoculation. *Autopsy.*—No muscle abscesses detected. Liver studded with minute yellow points. Mucous membrane of stomach very much reddened and eroded. *Cultures.*—Streptococci in pure culture and in large numbers from blood, liver, and spleen.
	15	7	54 c. c.	3 to 10 c. c.	Do.	1,200	1,110	+ Streptococci in blood and internal organs.	0	Died on day after the inoculation. *Autopsy.*—Liver contained psorosperm nodules, in which streptococci in large numbers were found along with the characteristic parasite. Kidneys pale; presented numerous minute cysts (?) over entire cortex. No abscesses anywhere. *Cultures.*—Streptococci in pure culture and in large numbers from blood, liver, spleen, kidney, and peritoneal cavity.
	14	12	109 c. c.	3 to 15 c. c.	Do.	1,000	1,040	+ Abscess in muscle; streptococci in blood.	+ (Stomach.)	Died on seventh day after inoculation. *Autopsy.*—A single abscess in muscle of the thigh was detected. Slight excess of moisture in peritoneal cavity. No abscesses in myocardium or kidneys. A widespread area of erosion and inflammation of the mucous membrane of the stomach. *Cultures.*—Streptococci in pure culture from spleen, liver, blood, kidney, and peritoneal cavity. They were present in only small numbers.
	15	13	124 c. c.	3 to 15 c. c.	Do.	1,440	1,420	+ Abscess on leg; streptococci in blood.	+ (Stomach.)	Died on eighth day after inoculation. *Autopsy.*—On right leg, near axilla, between skin and muscles, was a circumscribed collection of pus of about the size of an almond. No miliary abscesses detected. Several necrotic areas in liver. Mucous membrane of stomach very red and eroded. This animal had a very acute and painful erysipelas of the ear for several days after inoculation. *Cultures.*—Streptococci from blood, liver, and large abscess on leg.
	16	8	46·5 c. c.	3 to 10 c. c.	Do.	1,240	1,140	+ Muscle abscesses; streptococci in blood.	0	Died on second day after inoculation. *Autopsy.*—Muscle abscesses in left thigh and in abdominal muscles. Several abscesses in myocardium. *Cultures.*—Streptococci from blood, liver, kidney, and muscle abscesses.

Control Animals for this Experiment. These Animals were inoculated with the Same Material, at the Same Time, and in the Same Way as the Ten Preceding Animals. For Fifteen Days Each received 20 c. c. of Distilled Water daily, injected into the Stomach through a Soft-rubber Catheter.

Rabbits.	No. of days observed.	Total amount of alcohol received.	Daily dose of alcohol.	Interval between the beginning of alcohol and time of inoculation.	WEIGHT IN GRAMMES.		Lesions referable to inoculations. +=present. O=absent.	Lesions referable to the alcohol. +=present. O=absent.	Results and remarks.
					Beginning.	End.			
17	21	20 c. c. of water daily for 15 days. Alcohol was then begun. In 6 days 30 c. c. of alcohol given.	Water, 20 c. c. Alcohol, 10 c. c. 3 days, nothing.	5 days from the beginning of water. Inoculation same as preceding animals.	1,010	860	O (?) Condition of the ear of this rabbit was evidently erysipelatous. Unfortunately it was not examined at autopsy.	O	Died on fifteenth day after inoculation and on fifth day after alcohol was begun. *Autopsy.*—For five days before death this animal has had very sore ear. It was thick, painful, and dependent. Beyond a slight diminution in colour, internal organs were normal. No lesions of muscles. *Cultures*—Blood, liver, and kidneys sterile.
18	18	Water as in No. 17. One day, nothing; then got 10 c. c. alcohol on each of 2 days.	Water, 20 c. c. Alcohol, 10 c. c.	5 days from beginning of water. Inoculation as above.	1,130	1,020	O	O	Died after second dose of alcohol—*i. e.*, thirteen days after inoculation. *Autopsy*—Neither recent nor remote suppurative lesions anywhere. Small area of consolidation in left lung (inspiration pneumonia (?)); no bacteria found in cover slips from this. *Cultures.*—Blood, liver, and kidneys sterile.
19	52	No alcohol. 20 c. c. water daily for 15 days. Then got nothing for 37 days.	None.	5 days from beginning of water.	1,650	1,500	O	.....	The weight of the animal ran up as high as 1,710 and down as low as 1,450 grammes while in the experiment. At end of fifty-two days it was apparently well. Observation was discontinued. Showed no signs of sickness at any time.

20	16	Do.	"	Do.	1,120	1,130	0	.....	The weight of the animal went up to 1,200 grammes in course of the experiment. It was killed on sixteenth day. *Autopsy.*—Neither recent nor remote lesions anywhere. Viscera apparently normal. *Cultures.*—Blood, liver, spleen, and kidney sterile.
21	16	Do.	"	Do.	1,010	1,030	0	.....	Weight was up to 1,080 grammes at one time. Killed on sixteenth day. *Autopsy.*—No lesions of any kind anywhere. Animal apparently normal. *Cultures.*—Blood, liver, spleen, and kidney sterile.

Control Animals getting Nothing but the Inoculation.

22	11	.....	.....	No alcohol. Inoculated on same date and in same way as preceding animals.	920	960	+ Abscesses. Streptococcus septicæmia.	.....	Weight was up to 1,030 during experiment. Died five days after inoculation. *Autopsy.*—Abscesses in voluntary muscles, in myocardium, and in right kidney; none in left kidney. *Cultures.*—Streptococci in pure culture for blood, liver, spleen, kidney, and peritoneal cavity.
23	.....	.....	.....	.....	.....	.....	.....	.....	Intercurrent infection before it was inoculated. Excluded from the experiment.
24	25	.....	.....	Do.	1,320	1,040	+ (?) Caseous gland in axilla.	.....	Died on twenty-fifth day. For some days before death there was profuse watery discharge from mouth and nose. *Autopsy.*—A small caseous gland in right axilla. Did not contain streptococci on microscopic examination. No other lesions noted beyond numerous minute petechiæ over cortex of kidneys. *Cultures.*—Blood, liver, kidney, and peritoneal cavity sterile.
25	17	None until 16th day, when 1 dose of 10 c. c. was given.	.....	Do.	1,480	1,250	+ Abscesses in muscle.	.....	Died on second day after its dose of alcohol—*i. e.*, on twelfth day after inoculation. *Autopsy.*—Evidence of healed peritonitis. Two large abscesses in left pectoral muscle; no abscesses in myocardium or kidneys. The muscle abscesses were apparently of long standing. *Cultures.*—Streptococci from muscle abscesses. Blood and kidney sterile.

Rabbits.	No. of days observed.	Total amount of alcohol received.	Daily dose of alcohol.	Interval between the beginning of alcohol and time of inoculation.	WEIGHT IN GRAMMES. Beginning.	WEIGHT IN GRAMMES. End.	Lesions referable to inoculations. +=present. O=absent.	Lesions referable to the alcohol. +=present. O=absent.	Results and remarks.
26	16	No alcohol.	.....	Do.	1,490	1,090	O	.....	Killed on sixteenth day after experiment was begun—*i. e.*, eleventh day after inoculation. *Autopsy.*—Suppurative lesions, neither recent nor remote, were anywhere to be detected. *Cultures.*—No streptococci in either the blood or the internal organs.

Experiment III.

Table showing the Results of Intravenous Inoculation of Rabbits receiving Daily Doses of Ethyl Alcohol to the Stage of Intoxication, with Suspensions of Bacillus Coli Communis from Human Fæces.

27	6	55 c. c.	7·5 to 10 c. c.	5 days. Intravenous injection of 1 c. c. of opaque suspension of b. coli 52 hours old.	1,990	1,970	O	+ (Stomach.)	Died about five hours after inoculation. *Autopsy.*—Mucous membrane of stomach red; slight congestion of spleen and kidneys. *Cultures.*—A few colonies of bacillus coli from liver. Blood sterile.
28	6	55 c. c.	7·5 to 10 c. c.	Do.	1,890	1,800	O	O	Found dead on morning following the injection. *Autopsy.*—About 3 c. c. of reddish fluid in peritonæum. One or two dark areas in lungs. No other lesions. *Cultures.*—Liver, blood, and peritoneal cavity gave bacillus coli in pure culture.
29	23	240 c. c.	7·5 to 10 c. c.	Do.	1,400	920	O	O	Died on twenty-third day—*i. e.*, eighteen days after inoculation. *Autopsy.*—Pseudo-tuberculosis of apex of left lung. Liver pale and tough. Gall bladder distended with dark-green bile. *Cultures.*—Peritoneal cavity, five colonies, and bile, one colony; all were of bacillus coli.
30	6	55 c. c.	7·5 to 10 c. c.	Do.	1,690	1,770	O	O	Found dead on morning following the inoculation; had evidently been dead some time, as post-mortem changes were in progress in all the organs. *Cultures.*—Bacillus coli in pure culture from spleen, kidney, liver, and blood.

31	6	55 c. c.	7·5 to 10 c. c.	Do.	1,710	1,470	0	0	Found dead on morning following the inoculation. Post-mortem decomposition more or less advanced in all the viscera. No lesions discernible to naked eye. Pure cultures of bacillus coli obtained from blood, liver, spleen, kidney, and peritonæum.

The Following Animals were inoculated at the Same Time as the Preceding, with the Same Amount of the Same Material. They differed from the Preceding in having received Alcohol daily for a much Longer Time before the Inoculation.

32	32	112·5 c. c.	2·5 to 7·5 c. c.	31 days. Same inoculation as above.	1,130	1,070	0	+ (?) (Liver.)	This animal was so susceptible to alcohol that it could not be given regularly. Found dead on morning following the inoculation. The only lesions found at autopsy were very pale liver and a circumscribed œdematous patch in the lung. Colon bacilli in pure culture from blood, liver, spleen, and kidney.
33	75	440 c. c.	2·5 to 10 c. c.	33 days. Inoculated as above.	2,000	1,310	+ (Bile.)	0	Died on seventy-fifth day—*i. e.*, forty-three days after inoculation. *Autopsy.*—Moderate emaciation. Liver so thickly studded with coccidia that nothing could be detected as to presence of other lesions. Gall bladder very much distended with clear, pale bile in which floated amorphous flakes that could be seen through the walls of the organ. These consisted of leucocytes, epithelium, blood corpuscles, and bacteria. *Cultures.*—Pure cultures of bacillus coli from bile and blood. Cultures from liver contained colon bacilli with another bacillus (bacillus pyogenes).
34	88	580 c. c.	5 to 15 c. c.	Do.	1,560	1,330	+ (Liver and bile.)	0	Died on eighty-eighth day—*i. e.*, fifty-five days after inoculation. *Autopsy.*—Slight emaciation. Several irregular necroses in liver. Very great distention of gall bladder with clear, pale-greenish bile, in which was an amorphous sediment. *Cultures.*—Colon bacilli in pure culture from the bile. Liver and blood sterile.

Rabbits.	No. of days observed.	Total amount of alcohol received.	Daily dose of alcohol.	Interval between the beginning of alcohol and time of inoculation.	WEIGHT IN GRAMMES.		Lesions referable to inoculations. +=present. O=absent.	Lesions referable to the alcohol. -+=present. O=absent.	Results and remarks.
					Beginning.	End.			
35	87	560 c. c.	2·5 to 15 c. c.	Do.	1,620	1,220	O	+ (?) (Liver.)	Found dead on eighty-seventh day—*i. e.*, fifty-four days after inoculation. *Autopsy.*—Liver large, tough, rough on its surface. On section, the open mouths of vessels are very conspicuous. Gall bladder filled with normal dark-green bile. *Cultures* demonstrated the blood, liver, and bile to be sterile.
36	84	537·5 c. c.	2·5 to 10 c. c.	Do.	1,570	1,070	+ (Liver and bile.)	O	Found dead on eighty-fourth day—*i. e.*, fifty-one days after inoculation. *Autopsy.*—Both lungs in part affected with pseudo-tuberculosis. Liver presented several irregular parts of yellowish superficial necroses. *Gall bladder* very large, distended with pale, clear bile, in which floated flakes of amorphous material; these could be seen through the walls of the distended organ. *Cultures.*—Bacillus coli was obtained only from the bile. It was here in pure culture. From lungs bacillus pyogenes (?) was obtained in pure culture.

Controls. Injected at Same Time as the Others, but received no Alcohol.

37	79	.....	.....	Injected as above.	1,290	.....	.....	.....	Seventy-nine days after inoculation this animal is still alive. Had gained by sixtieth day 230 grammes. Observation discontinued.
38	79	.....	.....	" "	1,820	.....	.....	.....	Seventy-nine days after inoculation the animal is alive, is apparently well, though it had lost 200 grammes in weight by sixtieth day.

Experiment IV.

Table giving the Results of Intravenous Inoculation of Rabbits that were receiving Daily Doses of Ethyl Alcohol to the Stage of Intoxication, with Suspensions of Bacillus Coli Communis from Human Fæces.

39	11	90 c. c.	10 c. c.	Simultaneous. Inoculated with 0·5 c. c. of a suspension of bacillus coli from agar culture 24 hours old.	1,540	1,180	0	+ (Stomach.)	Died eight days after inoculation. Had been under observation for two days before any treatment was begun. *Autopsy.*—Acute fibrino-purulent pleuritis and pericarditis, due to intercurrent infection. Mucous membrane of stomach slightly eroded. No colon bacilli found in the cultures from blood and internal organs.
40	2	20 c. c.	10 c. c.	Do.	1,330	1,190	0	0	Died on the day following inoculation. *Autopsy.*—The only lesion found was an intussusception of the colon involving about 12 cm. of the length. It was very acutely congested, and evidently recent. *Cultures.*—Blood sterile. Liver: a few colonies of bacillus coli.
41	1	10 c. c.	10 c. c.	Do.	1,760	....	0	0	Died during night of the day on which it was inoculated. *Autopsy.*—No macroscopic lesions. *Cultures.*—Blood and liver contained large numbers of bacillus coli.
42	9	47·5 c. c.	5 to 7·5 c. c.	4 days. Inoculated as above.	1,180	950	0	+ (Stomach.)	Died on ninth day—*i. e.*, five days after inoculation. *Autopsy.*—Liver presented several areas of superficial necrosis. Gall bladder filled with normal bile. A recent volvulus, with intussusception of small intestine. Lymphatic apparatus of intestine enlarged, but not congested. Mucous membrane of stomach eroded. *Cultures.*—Colon bacilli in small numbers were obtained from liver, blood, bile, and spleen.
43	18	177·5 c. c.	7·5 to 10 c. c.	Do.	2,110	1,780	0	0	Died on eighteenth day—*i. e.*, fourteen days after inoculation. *Autopsy.*—Death ensued from intercurrent infection. No colon bacilli found in cultures.

Rabbits.	No. of days observed.	Total amount of alcohol received.	Daily dose of alcohol.	Interval between the beginning of alcohol and time of inoculation.	WEIGHT IN GRAMMES.		Lesions referable to inoculations. +=present. O=absent.	Lesions referable to the alcohol. +=present. O=absent.	Results and remarks.
					Beginning.	End.			
44	9	87·5 c. c.	7·5 to 10 c. c.	Do.	1,520	1,180	+ (Liver and bile.)	+ (Stomach.)	Died on ninth day—*i. e.*, five days after inoculation. *Autopsy.*—Moderate emaciation. Several irregular necroses in liver. Gall bladder distended with pale bile, in which amorphous flakes could be seen through the wall of the distended organ. Amorphous matter consisted of leucocytes, blood corpuscles, and bacteria. Mucous membrane of stomach red and eroded. *Cultures.*—Bacillus coli in blood, liver, bile, and spleen.

Controls; inoculated in the Same Way but with a Larger Dose of the Same Material as the Preceding Animals, but received no Alcohol.

45	.....	.....	.....	No alcohol. Intravenous inoculation of 0·8 c. c. of the same suspension used in preceding rabbits of this experiment.	1,560	.....	.....	.....	After forty-six days animal apparently well. Weight, 1,610 grammes.
46	.....	.....	.....		1,960	.....	.....	.....	After forty-six days animal apparently well. Weight, 1,840 grammes.
47	.....	.....	.....		1,510	.....	.....	.....	After forty-six days animal apparently well. Weight, 1,790 grammes.

Experiment V.

Table showing the Results of Intravenous Inoculation of Rabbits receiving Daily Doses of Ethyl Alcohol to the Stage of Intoxication, with Bouillon Cultures of Staphylococcus Pyogenes Aureus.

48	24	200 c. c.	10 to 15 c. c.	3 days. Intravenous inoculation of 0·3 c. c. of 24-hour bouillon culture of S. aureus. After 7 days, similar injection of 0·5 c. c.	1,900	1,250	+ (Kidneys.)	0	Died on twenty-fourth day—*i. e.*, twenty-one days after first and fourteen days after second inoculation. *Autopsy.*—Several old abscesses in the kidneys; gave appearance of being encapsulated; none in myocardium or voluntary muscles. Widespread pseudo-tuberculosis of right lung. This is evidently the cause of death. *Cultures.*—Staphylococcus aureus from kidney. Blood, liver, and bile sterile. A long, slender bacillus growing in felt-like, tenacious colonies from the diseased lung.
49	12	110 c. c.	10 c. c.	Do.	1,360	1,160	+ (Abscesses in kidneys and muscle.)	0	Died on twelfth day—*i. e.*, nine days after first and two days after second inoculation. *Autopsy.*—One small abscess in voluntary muscles. Numerous small abscesses in kidneys. None in myocardium. *Cultures.*—Staphylococcus aureus in very small numbers from blood and kidney. Peritonæum sterile.
				Controls.					
50	13	None until 7th day of experiment, then alcohol was begun. Total amount, 50 c. c.	10 c. c.	Alcohol given simultaneously with the second inoculation; first inoculation as above; second inoculation on seventh day consisted of 0·5 c. c. of a bouillon culture of S. aureus intravenously.	2,430	2,250	+ (Abscesses in kidneys.)	+ (Stomach.)	Died on thirteenth day—*i. e.*, ten days after first and six days after second inoculation. *Autopsy.*—Mucous membrane of stomach very much inflamed and eroded. Several conglomerate abscesses in kidneys, none in myocardium or in voluntary muscles. *Cultures.*—Staphylococcus aureus from kidney. Blood and liver sterile.

Rabbits.	No. of days observed.	Total amount of alcohol received.	Daily dose of alcohol.	Interval between the beginning of alcohol and time of inoculation.	WEIGHT IN GRAMMES. Beginning.	WEIGHT IN GRAMMES. End.	Lesions referable to inoculations. +=present. O=absent.	Lesions referable to the alcohol. +=present. O=absent.	Results and remarks.
51	4	None.	.....	One inoculation of 0·3 c. c. of bouillon culture of S. aureus intravenously. No alcohol.	1,170	920	+ (Abscesses in myocardium and muscles.)	O	Died four days after inoculation. *Autopsy.*—Abscesses in myocardium and kidneys. None in voluntary muscles. No cultures.

Experiment VI.

Table showing Results of Subcutaneous Inoculation of Rabbits receiving Daily Doses of Ethyl Alcohol to the Stage of Intoxication, with Cultures of Staphylococcus Pyogenes Aureus.

52	14	125 c. c.	10 to 15 c. c.	3 days. Subcutaneous inoculation of 1 c. c. of a 24-hour bouillon culture of S. aureus; 7 days later intravenous inoculation of 0·5 c. c. of similar culture.	1,350	930	+ (Disseminated miliary abscess formation.)	O	Died on fourteenth day—*i. e.*, eleven days after first and four days after second inoculation. *Autopsy.*—Typical disseminated miliary abscess formations in muscles and internal viscera. No cultures.
53	6	55 c. c.	7·5 to 10 c. c.	3 days. One subcutaneous inoculation as above.	1,380	1,080	+ (Site of inoculation.)	O	Died on sixth day—*i. e.*, three days after inoculation. *Autopsy.*—Small purulent mass at site of inoculation. No abscesses anywhere. Large part of both lungs affected with pseudo-tuberculosis. *Cultures.*—Staphylococcus aureus from site of inoculation. The slender bacillus referred to in protocol of Rabbit 48 obtained from lung.

Controls.

54	14	60 c. c. Alcohol was begun 7 days after first inoculation—*i. e.*, with the second inoculation.	10 c. c. for 6 days after second inoculation.	7 days after first inoculation; first inoculation was 1 c. c. of a bouillon culture of S. aureus subcutaneously; second was 0·5 c. c. of similar cultures intravenously.	2,390	2,080	+ (Abscesses in kidneys.)	+ (Stomach)	Died on fourteenth day—*i. e.*, seven days after second inoculation. *Autopsy.*—Post-mortem decomposition advanced. Straw-coloured fluid containing fibrin-like strands in pleural cavity. Abscesses in kidneys. Stomach injected. *Cultures.*—Staphylococcus aureus from kidney, liver, and pleural cavity. Bile, spleen, and peritonæum sterile.
55	8	No alcohol.	.....	First inoculation as above; second as above. No alcohol.	1,940	1,890	0	0	Died on day following last (second) inoculation. *Autopsy.*—Beyond a slight distention of the pericardial sac with fluid, there were no lesions anywhere to be detected by the naked eye. No abscesses anywhere. *Cultures.*—Staphylococcus aureus in moderate numbers from blood and kidney. Pericardial sac sterile.

Experiment VII.

Table showing Results of Intraperitoneal Inoculation of Rabbits receiving Daily Doses of Ethyl Alcohol, with Cultures of Staphylococcus Pyogenes Aureus.

56	4	40 c. c.	70 c. c.	3 days. Inoculated into peritonæum with 0·5 c. c. of 24-hour bouillon cultures of S. aureus.	1,050	880	0	0	Died on day following inoculation. *Autopsy.*—Beyond a few c. c. of reddish fluid in peritonæum, there was no evidence of peritonitis. No other lesions. *Cultures.*—Staphylococcus aureus in pure culture from peritonæum. Blood and liver sterile.

Rabbits.	No. of days observed.	Total amount of alcohol received.	Daily dose of alcohol.	Interval between the beginning of alcohol and time of inoculation.	WEIGHT IN GRAMMES.		Lesions referable to inoculations. +=present. O=absent.	Lesions referable to the alcohol. +=present. O=absent.	Results and remarks.
					Beginning.	End.			
57	12	110 c. c.	10 c. c.	3 days. First inoculation as in No. 56, second inoculation on seventh day. Intravenous injection of 0·5 c. c. of bouillon culture of S. aureus.	1,590	1,430	O	O	Died two days after second inoculation. *Autopsy.*—Small purulent nodule at point of puncture of first (intraperitoneal) inoculation. No peritonitis. Pericardium roughened, but contained no bacteria microscopically. It had not the appearance of recent inflammation. Minute red points over the surface of kidneys. No abscesses detected anywhere. *Cultures.*—A few colonies of staphylococcus aureus from blood and liver.
					Controls.				
58	3	.....	.....	No alcohol. Inoculated as No. 56.	.....	.....	O	O	Died on second day after inoculation. *Autopsy.*—Ruptured uterus, with three dead fœtuses lying in abdominal cavity. *Cultures.*—Blood sterile. Staphylococcus aureus and short bacilli in peritoneal cavity.
59	16	.....	.....	No alcohol. 7 days after the intraperitoneal inoculation an intravenous inoculation of 0·5 c. c. of bouillon culture of S. aureus was given.	1,000	870	+ (Abscesses in kidneys and in myocardium.)	O	Died nine days after second inoculation—*i. e.*, the intravenous injection. *Autopsy.*—Kidneys and myocardium studded with abscesses. Lung presented several caseous or suppurating nodules that contained cocci. *Cultures.*—Staphylococcus aureus in liver, kidney, and bile.

Experiment VIII.

Table showing Results of Intravenous Inoculation of Rabbits receiving Alcohol daily to the Stage of Intoxication, with Bouillon Cultures of Staphylococcus Pyogenes Aureus.

60	2	12·5 c. c.	12·5 c. c.	Simultaneous. Intravenous inoculation with 0·4 c. c. of a 24-hour bouillon culture of S. aureus.	.....	.....	+ (Heart and kidneys.)	+ (Stomach.)	Died on second day. *Autopsy.*—Very delicate network of lymph over surface of heart. Numerous *very* minute yellow points in kidneys. Mucous membrane of stomach red and eroded. *Cultures.*—Staphylococcus aureus in small numbers from blood, in very large numbers from kidney.
61	3	22·5 c. c.	10 to 12·5 c. c.	Do.	1,990	1,860	+ (Myocardium, kidneys.)	+ (Stomach.)	Died on third day. *Autopsy.*—Animal died as result of an accident. A small amount of alcohol was inspired into lungs. Death followed at once. Several yellow points in myocardium, and numerous similar points in kidneys. None seen in voluntary muscles. Mucous membrane of stomach very red and eroded over large part of its surface. *Cultures.*—Blood sterile. Kidneys gave staphylococcus aureus in very large numbers.
62	1	12·5 c. c.	.....	Do.	2,000	.....	0	+ (Stomach.)	Died fifteen hours after inoculation. *Autopsy.*—Entire cardiac two thirds of stomach intensely reddened and mucous membrane eroded. *Cultures.*—Blood sterile. Kidney, four colonies of staphylococcus aureus.
63	2	22·5 c. c.	10 to 12·5 c. c.	Do.	2,050	.....	+ (Kidneys.)	+ (Stomach.)	Died forty-eight hours after inoculation. *Autopsy.*—Conglomerate and single miliary abscesses in both kidneys. Walls of stomach very much thickened, œdematous, and acutely reddened over about half its surface. Stomach contained small amount of dark grumous material. *Cultures.*—Blood sterile. Kidney contained staphylococcus aureus.
64	1	12·5 c. c.	.....	Do.	1,390	.....	+ (?) (Pericardium.)	0	Found dead fifteen hours after inoculation. *Autopsy.*—Pericardium filled with bloody serum Small amount of fluid in peritonæum. *Cultures.*—Blood, twenty-one colonies. Kidney, one colony of staphylococcus aureus.

Rabbits.	No. of days observed.	Total amount of alcohol received.	Daily dose of alcohol.	Interval between the beginning of alcohol and time of inoculation.	WEIGHT IN GRAMMES. Beginning.	WEIGHT IN GRAMMES. End.	Lesions referable to inoculations. +=present. O=absent.	Lesions referable to the alcohol. +=present. O=absent.	Results and remarks
65	1	12·5 c. c.	.....	Do.	1,790	.....	O	+ (Stomach.)	Found dead fifteen hours after inoculation. *Autopsy.*—Small amount of fluid in peritonæum. No peritonitis. Mucous membrane of stomach red and eroded. *Cultures.*—Staphylococcus aureus in blood and kidneys.
				Controls—inoculated, but received no Alcohol.					
66	11	.....	.....	No alcohol. Inoculated as above.	2,150	1,530	+ (Abscesses in muscles, kidneys, and joint.)	.....	Died on twelfth day after inoculation. *Autopsy.*—Numerous old abscesses in muscles, many almost as large as a pea. Purulent suppuration about the joint of right hock. Numerous old abscesses in kidneys. None in heart. *Cultures.*—Staphylococcus aureus from kidney and suppurating joint. Blood sterile.
67	1	.....	.....	Do.	2,110	.....	O	.....	Died twenty hours after inoculation. *Autopsy.*—Numerous extremely minute white points in myocardium. Several pale, circumscribed, and also diffuse points in kidneys. Mucous membrane of stomach normal. *Cultures.*—Blood and kidneys sterile.
68	1	.....	.....	Do.	1,720	.....	+ (Heart, muscles, kidneys.)	.....	Died twenty-one hours after inoculation. *Autopsy.*—Pericardium distended with serum. Heart covered with a delicate network of fibrin. Myocardium studded with minute pale points. Voluntary muscles presented numerous small lens-shaped pale points. Kidneys presented numerous pale areas that dipped into the cortex in a wedge-shaped manner. *Cultures.*—Staphylococcus aureus from kidney. Blood sterile.

69	36	.....	.....	Do.	1,730	.....	.....	.....	After forty-three days animal is apparently well. Weighs 1,560 grammes.
70	1	.....	.....	Do.	1,350	.....	+ (?) (Pericardium.)	.....	Died twenty hours after inoculation. *Autopsy.*—Pericardium filled with serum, in which was a colourless clot and a few strands of fibrin. Diffuse pale areas in cortex of kidneys. *Cultures.*—Blood, one colony of staphylococcus aureus. Kidney sterile.
71	4	.....	.....	Do.	1,970	1,780	+ (Abscesses in heart, kidneys, and muscles.)	.....	Died on fifth day after inoculation. *Autopsy.*—On inner aspect of left thigh was a collection of pus about the size of a chestnut. Numerous abscesses in muscles. Several abscesses in myocardium. Numerous abscesses in both kidneys. Small area of peritonitis in left iliac region. *Cultures.*—Staphylococcus aureus from blood, kidney, and abscess in thigh.

Experiment IX.

Table showing the Results of Subcutaneous Inoculation of Rabbits receiving Alcohol to the Stage of Intoxication daily, with Cultures of Staphylococcus Pyogenes Aureus.

72	11	55 c. c.	5 c. c.	Simultaneous. Subcutaneous inoculation of 1 c. c. of a bouillon culture of S. aureus.	2,520	2,140	+ (Site of inoculation.)	0	Died on twelfth day after inoculation. *Autopsy.*—At site of inoculation in the left groin the tissues were dissected apart, leaving a moderately large excavation. This contained neither serum nor pus. About the borders of this the tissues were very œdematous, the œdema extending up into the neck and into the thighs of both sides. Middle lobe of right lung consolidated. *Cultures.*—Staphylococcus aureus from blood, from site of inoculation in large numbers, and from kidney. Lung also contained them, but associated with a short bacillus.
73	14	75 c. c.	5 to 10 c. c.	Do.	1,690	1,460	+ (?) (Pericarditis and pleuritis.)	0	Died on fifteenth day after inoculation. *Autopsy.*—A trace of suppuration only at site of inoculation. Fibrino-purulent pleuritis and pericarditis. Moderate amount of blood-stained purulent matter in pleural cavities. *Cultures.*—From pleural cavity a feebly growing staphylococcus was obtained. Can not say that it was or was not staphylococcus aureus.

Rabbits.	No. of days observed.	Total amount of alcohol received.	Daily dose of alcohol.	Interval between the beginning of alcohol and time of inoculation.	WEIGHT IN GRAMMES.		Lesions referable to inoculations. +=present. O=absent.	Lesions referable to the alcohol. +=present. O=absent.	Results and remarks.
					Beginning.	End.			
74	5	30 c. c.	5 to 10 c. c.	Do.	1,720	1,400	+ (?) (Pericarditis and pleuritis.)	O	Died on fifth day as direct result of last dose of alcohol, from under the influence of which it did not recover. Animal was very susceptible to alcohol. By mistake its last dose was 10 c. c. instead of 5 c. c., as had been the case. *Autopsy.*—Slight purulent reaction at site of inoculation. Purulent pericarditis and pleuritis. No abscesses. Slight peritonitis. *Cultures.*—Blood, left pleura, kidney, and spleen gave a feebly growing staphylococcus identical with that found in No. 73. Site of inoculation gave typical staphylococcus aureus.
75	1	10 c. c.	.....	Do.	1,590	.... ..	O	O	Died during night of day of inoculation. *Autopsy.*—No macroscopic lesions anywhere to account for death. Stomach normal and filled with partly digested food. *Cultures.*—Staphylococcus aureus from site of inoculation. Blood and kidney sterile.
76	14	140 c. c.	10 c. c.	Do.	2,420	2,050	+ (?) (Pericarditis and pleuritis.)	O	Died on fifteenth day after inoculation. *Autopsy.*—At site of inoculation was circumscribed collection of cheesy pus of about size of an almond. Fibrino-purulent pericarditis and pleuritis. Both thoracic cavities filled with purulent matter. *Cultures.*—Staphylococcus aureus in large numbers from the site of inoculation. A few colonies of cocci from liver. Thoracic cavity gave large numbers of slowly growing micrococci that gave the impression of an enfeebled culture of staphylococcus aureus (see Nos. 73 and 74).
77	9	90 c. c.	10 c. c.	Do.	1,660	1,260	O	O	Died on tenth day after inoculation. *Autopsy.*—Only slight reaction at site of inoculation. No lesions on macroscopic examination to account for death. *Cultures.*—Blood, liver, and kidney sterile.

Controls.

78	46	.....	.....	No alcohol. Inoculated as above.	2,130	.....	0	.....	Apparently well after forty-six days. Weighs 1,850 grammes.
79	46	.....	.....		1,560	.....	0	.....	Apparently well after forty-six days. Weighs 1,780 grammes.
80	46	.....	.....		1,490	.....	0	.....	Apparently well after forty-six days. Weighs 1,570 grammes.

Experiment X.

Results of Intravenous Inoculation of Rabbits that were receiving Alcohol daily to the Stage of Intoxication, with a Culture of Staphylococcus Pyogenes Aureus attenuated (?) by Exposure to Chloroform.

81	.7	45 c. c.	5 to 7·5 c. c.	2 days. Intravenous inoculation with 0·6 c. c. of a chloroformed culture of S. aureus.	1,200	1,050	+ (Pelvis of kidney. Thoracic cavity (?).)	+ (Stomach.)	Died on fifth day after inoculation. *Autopsy.*—Pleural cavities and pericardium filled with clear serum. Purulent inflammation of pelvis of right kidney; no cortical lesions. Urine contained microscopically numerous micrococci. Mucous membrane of stomach eroded. *Cultures.*—Blood, left kidney, peritonæum, and liver sterile. Pelvis of right kidney and urine contained staphylococcus aureus in large numbers.
82	4	22·5 c. c.	5 to 7·5 c. c.	Do.	1,630	1,640	+ (?) (Peritonitis.)	+ (Stomach.)	Died on second day after inoculation. *Autopsy.*—General acute peritonitis, with deposit of lymph over coils of intestines. Mucous membrane of stomach very red and eroded. *Cultures.*—Blood, kidney, and peritonæum all contained a pure culture of a staphylococcus that grew slowly and suggested an attenuated staphylococcus aureus.

Controls.

83	28	.....	.....	No alcohol. Inoculated as above.	1,350	1,530	.....	.....	The observation was discontinued at twenty-eighth day. The animals had gained in weight, and had at no time shown effects of the treatment.
84					1,410	1,750			

EXPERIMENTAL CONTRIBUTION TO THE STUDY OF THE PATH BY WHICH FLUIDS ARE CARRIED FROM THE PERITONEAL CAVITY INTO THE CIRULATION.

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Introductory.

Though it has been recognised since the experiments of Magendie that fluids within the lymph spaces may enter the circulation directly through the walls of the blood vessels, the acceptation of this fact did not carry with it any serious doubt with regard to the validity of the general belief that, as a rule, it is the task of the lymphatics to carry the lymph from the lymph spaces, including the serous cavities, through the lymphatic ducts into the circulation.

Lately, however, a number of writers, stimulated by the well-known labours of Heidenhain on the secretion of lymph * and on the mode of absorption from the intestines, † have been led by their experiments to the rather remarkable conclusion that the absorption of fluids from serous cavities, especially from the peritoneal cavity, is in the main carried out by the blood vessels, the capillaries within the lymph spaces, while the lymphatics have at the utmost only a minor share in this task. We shall abstain from going over the entire literature of this subject, which is rather extensive, but shall confine our introductory remarks to a brief reference to that of more recent date, especially to the writings of Starling and Tubby, Orlow and Heidenhain, and Hamburger. These writers, though following quite dif-

* Heidenhain, Versuche u. Fragen zur Lehre von der Lymphbildung. Pflüger's *Archiv für die gesammte Physiologie*, Bd. xlix, p. 209.

† Heidenhain, Neue Versuche über die Aufsaugung im Dünndarm. Pflüger's *Archiv*, Bd. lvi, p. 579.

ferent lines of experiment, arrived independently of one another at the above-mentioned conclusion, each method furnishing a new and apparently well-supported argument in its favour.

The experiments of Starling and Tubby * consisted in introducing certain staining fluids into the abdominal cavity and noting the exact time of their appearance in the urine and in the flow of lymph from the thoracic duct. They state that the colour appears much earlier in the urine than in the lymph, a fact which can be explained only by the assumption that the absorption takes place through the blood vessels. They are even in doubt whether the slight tinting of the lymph is not due to a resecretion from the blood.

Orlow † and Heidenhain ‡ introduced a measured quantity of fluid into the abdominal cavity of dogs and removed it again after a few hours. They found that invariably a more or less large amount of the fluid was absorbed during this time, while the flow of lymph from the thoracic duct, which was measured before the introduction and after the withdrawal of the fluid, remained the same. This, again, would go to show that the fluid absorbed did not make its way through the thoracic duct. Cohnstein,# who confirms this statement, claims nevertheless that there is a difference in amount in the flow of the lymph after introduction of fluid into the abdomen, inasmuch as in a dog tied on a holder it is constantly diminished. It is obvious, however, that this slight difference is entirely out of proportion to the quantity absorbed from the peritoneal cavity. ||

Hamburger, △ finally, studied the effect of ligation of the left innominate vein, which means also the ligation of the thoracic duct, upon the absorption of fluids of different osmotic pressures, introduced

* E. H. Starling and O. H. Tubby, On Absorption from and Secretion into the Serous Cavities. *Journal of Physiology*, vol. xiv, p. 140.

† Orlow, Einige Versuche über die Resorption in der Bauchhöhle. Pflüger's *Archiv*, Bd. lix, p. 170.

‡ Heidenhain, Pflüger's *Archiv*, Bd. lxii, p. 320.

Cohnstein, Ueber Resorption aus der Peritonealhöhle. *Centralblatt für Physiologie*, Bd. ix, No. 13 (Sept. 21, 1895).

|| See Hamburger, *Centralblatt für Physiologie*, Bd. ix, No. 16 (Nov. 2, 1895).

△ Hamburger, Ueber die Regelung der osmotischen Spannkraft von Flüssigkeiten in Bauch- und Pericardialhöhle. Du Bois-Reymond's *Archiv für Physiologie*, 1895, p. 281.

into the abdominal cavity of rabbits, and comes to the conclusion that the lymphatics assist but little in the process of absorption.

Experiments with Small Quantities.

Without entering for the present into a discussion of the merits of these arguments, we wish first to report a set of experiments on absorption from the peritoneal cavity which, in opposition to those of the above-quoted authors, show, as we believe, unmistakably that the lymph is carried into the circulation more readily and much earlier by the way of the lymphatics than directly through the walls of the blood vessels. It should, however, be stated at the outset that there is one important point in which the method adopted in the experiments of the other authors differed from that employed in our research. We introduced into the abdominal cavities of rabbits mostly very small quantities, not more than 1 or 2 cubic centimetres of the fluids being employed; the absorption of such small amounts certainly afforded a greater similarity with that of the normal lymph than when the large quantities of fluids used by these other writers were employed.

Substances which produce characteristic manifestations after passing the circulation were introduced, after which the first appearance of these manifestations was noted. Thus after strychnine had been injected we watched for the appearance of the outbreak of the characteristic tetanus, or when potassium ferrocyanide had been introduced the exact time of its first appearance in the urine was duly recorded. This was done in normal animals as well as in animals with ligated lymphatic ducts and the times of the first appearance of the appropriate manifestations in the animals of both series were compared. As we were compelled for various reasons to confine our study exclusively to rabbits we did not attempt to ligate the thoracic duct itself in these animals, but resorted to the method of ligating the left innominate vein just below the juncture of the subclavian and the jugular veins. Ligation at this point serves practically the same purpose, since it entirely prevents the lymphatic duct from emptying its contents into the circulation. This same method was also employed independently by Hamburger, as stated above, but, in justice to ourselves, it should be

stated that these experiments of ours were started months before the publication of Hamburger's work. For reasons to be explained hereafter, in most of our experiments we ligated also the right innominate vein, and thus prevented also the lymph carried by the right lymphatic duct from entering the circulation.

The ligation of both innominate veins, however, not only effects an exclusion of the lymph from the circulation, but also prevents the return of the venous blood from the brain, and indeed certain of our experiments seemed to show that the venous hyperæmia of the brain exerts an influence upon the tetanic outbreak as well as upon the secretion of the urine. If, therefore, after ligation of both innominate veins some alteration in the ordinary manifestations occurs, it might be urged that these differences can just as well be ascribed to the venous hyperæmia as to the ligation of the lymphatics. To meet this objection we ligated in each control rabbit both external jugular veins, which in rabbits are the main carriers of the venous blood from the brain, the internal jugular veins being very small. In this way the rabbits in both series were put upon an equal footing with regard to the state of venous hyperæmia of their brains, but still differed with regard to their lymphatic ducts, which were open in those of the one and ligated in those of the other series. Hence we were justified in considering any variations in the manifestations in the two rabbits as being due to the difference in the state of the lymphatics.

The animals were well anæsthetized, the anæsthetic used in this set of experiments being for the most part ether, as we had occasion to learn that chloral retards or even suppresses the outbreak of the strychnine tetanus. The strychnine, as well as the potassium ferrocyanide, was mostly dissolved in a one-per-cent solution of sodium chloride, which is nearly isotonic with the serum of the rabbit. The results, however, were exactly the same when the saline solution had a hypotonic concentration of 0.6 or of 0.3 per cent.

Our procedure was then mostly as follows: Two rabbits of about the same weight and at about the same stage of digestion were tied on holders and anæsthetized. In one of the animals the innominate veins were dissected out and threads put around them. This rabbit we con-

stantly termed A. In the other rabbit, which we termed B, threads were put around the external jugular veins. The several veins in both rabbits were then ligated simultaneously. Very small incisions were next made in the linea alba leading to the peritoneal cavity. Through these fine openings a fixed amount of the fluid was introduced by means of a syringe with a blunt point, the openings being clamped after each injection, thus preventing the escape of any portion of the fluid. If, however, by any mischance a droplet did escape or was wasted, this difference was always charged to A. For the same reason the injection was always first made into A in order to give to it the benefit of the extra time which elapsed between the two injections. The urine was obtained by pressing over the region of the bladder, which very rarely failed to respond. This method was the more satisfactory, since with even the smallest droplet of urine diluted with water it was easy to demonstrate the presence of potassium ferrocyanide by means of the characteristic Prussian-blue reaction. Even in a dilution of 1 to 40,000 the addition of perchloride of iron brings out this fine blue colour.

We shall now give condensed protocols of a few of our experiments which speak for themselves:

Experiment 71.—Rabbit A, 1,750 grammes, both innominate veins ligated. Rabbit B, 1,750 grammes, both external jugular veins ligated. Injected into the abdominal cavities of each 0.6 milligramme of strychnine at 4.19 P. M. No effect. At 4.29, 0.3 milligramme of strychnine was injected again into each; eight minutes later, at 4.37, B had a characteristic opisthotonos; at 4.44, no convulsions yet in A; added 0.3 milligramme of strychnine; at 5.3, added again 0.4 milligramme of strychnine; characteristic tetanus in A at 5.20. This means that in the rabbit without the lymphatics the strychnine took effect forty-three minutes later than in the normal rabbit, and that with a dose nearly twice as large.

Exp. 75.—A, female rabbit, 1,460 grammes, both innominate veins ligated. B, female rabbit, 1,430 grammes, both external jugular veins ligated. Injected at 4.59 P. M. into the abdominal cavity of each rabbit one cubic centimetre of a five-per-cent solution of potassium ferrocyanide. Test of urine after ten minutes negative in both. At 5.18 injected again into both 0.5 cubic centimetre of same fluid. At 5.29, Prussian-blue reaction in the urine of B; the urine of both tested every ten minutes; B

retained the positive reaction. Urine of A shows for the first time the reaction of Prussian blue at 6.6. This means that in the rabbit without the lymphatics the potassium ferrocyanide reaches the urine thirty-seven minutes later than in the other rabbit. The same rabbits were then used for an experiment with strychnine. At 6.14, 0.8 milligramme of strychnine was injected into each; B succumbed to a typical tetanus at 6.22; no convulsions in A. At 6.44 again 0.8 milligramme of strychnine injected into A. Tetanus at 6.55. The second part of the experiment gave results confirmatory of the first.

Exp. 76.—A, male rabbit, 1,266 grammes, both innominate veins ligated. B, female rabbit, 1,280 grammes, both external jugular veins ligated. At 4.30 injected into A 0.9 cubic centimetre of five-per-cent potassium ferrocyanide, and at 4.33 injected into B 0.7 cubic centimetre of same solution. At 4.43 no reaction in the urine of either. At 4.48 injected again into each 0.5 cubic centimetre of same solution; at 5.17 Prussian-blue reaction in the urine of B, absent in the urine of A. Urine of A tested every ten minutes. Till 6.14 no reaction. At 5.35 a piece of absorbent cotten pressed into the right thoracic aperture in A near ligature of right innominate vein, where some drops of lymph seemed to collect, showed the Prussian-blue reaction. No reaction in left aperture, where the tissue seemed to be dry. At 6.18 the testing of urine was discontinued and rabbits were used for strychnine—large doses. Again a difference in favour of B, though not as great as before.

Autopsy of A: Distinct reaction of Prussian blue all over the abdominal cavity, except within the bladder. Autopsy of B: No reaction of Prussian blue in the abdominal cavity; present in the urine. In this experiment it looks as if very little, if any, absorption had taken place in rabbit A, with the ligated lymphatics.

Exp. 77.—Rabbit A, 1,140 grammes, both innominate veins ligated. Rabbit B, 1,080 grammes, both external jugular veins ligated. At 4.41 injected into A 0.9 cubic centimetre of five-per-cent potassium ferrocyanide, and into B 0.7 cubic centimetre of same fluid. At 4.52 test of urine of both negative. At 4.55 injected again into A 0.6 cubic centimetre, and into B 0.5 cubic centimetre of same fluid. At 5.37 the urine of B showed positive reaction of Prussian blue. Testing urine of A every ten minutes continued until 6.17, but no reaction. At 5.55 there was a positive reaction on cotton dipped in right aperture of A; no reaction from left. At 6.30 animals used for strychnine with same results as in the other experiments.

Exp. 84.—Rabbit A, 2,180 grammes, both innominate veins ligated. Rabbit B, 2,150 grammes, both external jugular veins ligated. Both

holders kept slanting; heads higher. At 3.56 injected into each 1 cubic centimetre of potassium ferrocyanide. Urine tested every ten minutes. At 4.20, in the urine of B, a positive Prussian-blue reaction; testing continued every ten minutes. At 4.55 first positive reaction in urine of A. Right thoracic aperture shows positive reaction of Prussian blue at 4.30; no reaction in left aperture. At 5.20 animals used for an experiment with strychnine, with the same characteristic results as those reported above.

These experiments go to show that, in the first place, in two rabbits of about the same size an equal dose of strychnine produced a tetanic outbreak much earlier in the animal with open lymphatics than in the one whose lymphatic ducts were ligated. Moreover, some of these control rabbits, as we know from experiments not mentioned here, did not show any signs of tetanus at all when no more strychnine was injected into them. It is true that when the initial doses of strychnine were large the difference between the results which occurred in A and B was less striking, and it could probably be proved that within certain limits the interval between the tetanic outbreaks in A and B increases in inverse proportion to the doses used for injection. For the purpose of establishing the presence of such a difference between the two kinds of rabbits, we have found it advisable, therefore, to begin the injections with small doses, which were followed from time to time by still smaller doses, until the first tetanic outbreak in one of the rabbits occurred. Disregarding, then, for the present, the question of the influence of the quantity injected upon the absorption, it must be conceded that our experiment goes some way toward establishing the fact that the rabbits with open lymphatics absorb strychnine from the peritoneal cavity a great deal more readily than those whose lymphatics have been rendered impassable.

It must be conceded, however, that the experiments with strychnine by themselves cannot be considered as furnishing an unassailable proof for the conclusion that peritoneal fluids are carried into the circulation mainly by the lymphatics. Against such an assumption it might be urged that rabbits of the same size and apparently in the same general condition show quite a different degree of irritability

and react differently to the same doses of strychnine, so that an effective dose for one rabbit is often subminimal for another of the same size. Sometimes it seemed to us that by the indolence or hyperexcitability of a rabbit we could predict whether it would require a large or a small dose to produce a tetanic effect; and indeed we have often taken the point of excitability of the animals into consideration when comparing the effect of strychnine upon two rabbits. Now, as a matter of fact, we never saw a rabbit with ligated lymphatic ducts succumbing sooner to the effect of strychnine than a normal one. Nevertheless it might be claimed that, after all, chance had played a trick upon us, giving us constantly indolent rabbits for series A and irritable rabbits for series B, so that the difference of time between the tetanic outbreak might be due only to this idiosyncrasy of the rabbit, and not to the ligation of the lymphatics. This is, to be sure, not a very probable assumption, but it suffices nevertheless to make the demonstration appear incomplete.

The complete proof, however, of the importance of the part played by the lymphatics in the process of absorption from the peritoneal cavity is furnished, as we think, by the experiments with the injections of potassium ferrocyanide. We see in these the Prussian-blue reaction appearing in the urine of the rabbit with the ligated lymphatics (A) much later than in B, with the lymphatics open and unobstructed. This was a constant occurrence in all our experiments made with potassium ferrocyanide, and the delay in A was here even more striking than in the experiments with strychnine, the reaction sometimes not appearing at all during the entire time devoted to the observation. Now in these experiments with potassium ferrocyanide there seems to be no possible ground for assuming that constitutional variations with regard to the secretion of the urine were the cause of the delayed reaction. Moreover, the same rabbit with ligated lymphatics in which a late appearance of potassium ferrocyanide in the urine was noted shows also a retardation of the tetanic outbreak after injection of strychnine; one could hardly go so far as to claim this constant coincidence as merely accidental. Finally, the autopsy in Experiment 76, made two hours after injection, does away with all

doubts. In the rabbit with the ligated lymphatic duct (A) Prussian-blue reaction could be obtained all over the peritoneal cavity, and no reaction was present in the fluid within the bladder, while in the rabbit with open lymphatic ducts (B) no reaction could be obtained in the abdominal cavity and a positive reaction was present in the fluid in the bladder. This finding certainly means that with open lymphatics there is excellent absorption; with ligated lymphatics there is no absorption, or, if it occurs at all, it is very slow.

To complete our experience with the injections of potassium ferrocyanide we will append a few short protocols of experiments in which only one lymphatic duct was ligated:

Exp. 81A.—Male rabbit, 1,920 grammes, right jugular vein ligated. At 9.37 P. M. injected into peritoneal cavity 1 cubic centimetre of potassium ferrocyanide; at 9.59, twenty-two minutes after injection, Prussian-blue reaction in the urine.

Exp. 81B.—Female rabbit, 2,100 grammes, right innominate vein ligated. At 10.29 P. M. injected into peritoneal cavity 1 cubic centimetre of potassium ferrocyanide. At 10.44, 0.5 cubic centimetre of same solution was added. First reaction of Prussian blue in urine appeared at 11.20 P. M., fifty-two minutes after first injection and thirty-two minutes after second injection.

Exp. 81C.—Male rabbit, 1,560 grammes, left innominate vein ligated. At 9.58 P. M. injected into the peritoneal cavity 1 cubic centimetre of five-per-cent potassium ferrocyanide; urine tested every ten minutes. First Prussian-blue reaction at 11.8, seventy minutes after injection.

Exp. 83A.—Male rabbit, 1,430 grammes, both jugular veins and left innominate vein ligated, thoracic duct apparently torn, whitish fluid oozing. At 5.40 P. M. injected 1 cubic centimetre of five-per-cent potassium ferrocyanide; at 5.51 P. M. injected an additional 0.6 cubic centimetre of same solution. The whitish fluid in the left aperture showed at 5.50 a positive Prussian-blue reaction. At 6.34 the first positive Prussian-blue reaction appeared in the urine, fifty-three minutes after first and forty-three minutes after second injection. Lymph of thoracic duct showed Prussian-blue reaction after injection.

Exp. 83B.—Male rabbit, 1,160 grammes, both jugular veins and right innominate vein ligated. Injected into peritoneal cavity at 5.40 P. M. 1 cubic centimetre of potassium ferrocyanide. At 5.51 injected an additional 0.6 cubic centimetre of same solution; first Prussian-blue reaction

in urine at 6.18, thirty-eight minutes after first and twenty-seven minutes after second injection.

These experiments show clearly that the ligation of even one lymphatic duct retards distinctly the time of the first appearance of potassium ferrocyanide in the urine, and it is quite remarkable to find that the ligation of the right lymphatic duct also exerts a marked influence upon the absorption from the peritoneal cavity, although this influence is apparently less than that exerted by the left lymphatic duct. Although our experiments in this regard are not as yet numerous enough to satisfy us as to the conclusions which may safely be deduced from them, they certainly support those which have already been drawn from the other experiments and explain at the same time our motive for ligating both innominate veins when proceeding to show in a general way the importance of the lymph paths for the absorption of fluids from the peritoneal cavity.

Our experiments with the ligation of the innominate veins show that ligation of the lymphatic ducts retards the entrance of the fluids from the peritoneal cavity into the circulation. But they also show that the ligation merely retards, but does not prevent the final entrance of the fluid into the circulation. On the contrary, in all cases in which our time and patience permitted a sufficiently long observation, the Prussian-blue reaction finally appeared even in the animals both of whose lymphatic ducts were ligated. The final appearance of the reaction in the urine under these circumstances can not be otherwise explained than by the assumption that the potassium ferrocyanide in the abdominal cavity made its way into the circulation directly through the walls of the blood vessels.

We would insist, however, that this fact can not by any means be urged as a proof for the assumption that in normal animals also the lymph passes into the circulation not only by way of the lymphatics, but also directly through the blood vessels. One of the main causes for the lymph secretion, and certainly for the lymph movement, is to be found in the difference between the intra- and extra-capillary pressure. The pressure in the capillaries is higher than in the surrounding lymph spaces; in the lymph spaces it is higher than in the

lymph vessels; and in these, again, it is higher than in the large ducts; finally, the pressure in the lymphatic ducts is higher than in the innominate veins. Now, while it is only natural that the fluid in the lymph spaces should move through the lymphatics to a locality of the circulation where the pressure is lower, it is against reason to expect that the fluid shall move directly in opposition to an increase of pressure—viz., from the lymph spaces into the capillaries.

This rule, however, applies only to normal animals, and not to animals whose lymph ducts have been ligated. Immediately after the outflow of the lymph into the circulation is prevented the pressure within the ligated duct begins to rise; this increase is transferred backward toward the lymph spaces, and the longer the ligation lasts the higher the pressure rises all along the lymph vessels and the lymph spaces. Thus it is probable that after a certain length of time the pressure within all the lymph spaces becomes equal to the pressure within the blood capillaries, and in some lymph spaces the pressure of the fluid within them becomes even higher than that in the blood capillaries around which they are collected and into which they now discharge, impelled by the same force by which the lymph usually leaves the capillaries—by filtration. Now we have seen in our experiments that even under such favourable circumstances and with no other road open the filtration of the fluid from the lymph spaces into the blood vessels occurs somewhat slowly. How can it be expected, then, that in normal animals, where the filtration against pressure is impossible and where by the way of open lymphatics there is an easy path by which the fluid can move on, this fluid should nevertheless select its way through the walls of the blood vessels?

Before concluding this part it may be added that Cohnstein,* who is also fighting against the degradation of the lymphatics, nevertheless admits that colouring substances, hyper- and hypo-tonic salt solutions, and other "different" fluids actually enter the circulation through the walls of the capillaries. We have experimented with a five-per-cent solution of a salt—potassium ferrocyanide—which is

* Cohnstein, loc. cit.

certainly a hypertonic fluid with a high osmotic pressure and should therefore have met with no difficulty in entering into the circulation directly through the walls of the blood vessels by the simple process of osmosis. Nevertheless we have seen that our solution of this salt preferred the track of the lymphatics.

Experiments with Larger Quantities.

Among the above-mentioned writers on this subject, Hamburger * holds the most decided views as to the secondary rôle played by the lymphatics in the act of absorption of fluids from the peritoneal cavity. Hamburger's opinion is founded chiefly upon the results of his own experiments. Some months previous to the publication of his article we started a series of experiments which were, so far as the question of absorption from the peritoneal cavity is concerned, quite similar to those of Hamburger, but which do not by any means bear out the conclusions at which this author arrived. Before entering, however, upon the report of our own experiments we wish, on account of the importance of the subject, to adduce a few reasons to show that, in our opinion, Hamburger's own experiments do not justify his far-reaching conclusions. In the first place, the method employed by Hamburger is not adapted to furnish precise results. In nearly all the experiments which have a bearing on the present question he introduced the fluid into the abdominal cavity through a sharply-pointed canula which was inserted into the lumbar region of the rabbit, and removed the fluid again by inserting a wider trocar. Our own experience has confirmed the assertion made by many writers before us, that in plunging a sharp canula into the abdomen, in many cases the needle gets into the intestinal lumen. This happens more especially when working with rabbits. Furthermore, the inserted trocar certainly does not remove all the fluid from the abdomen, and, despite the assertions of Hamburger, we must affirm that our experience has shown to us that by this method sometimes even more than half of the quantity remains in the abdomen. Under such circumstances

* Hamburger, Du-Bois-Reymond's *Archiv*, 1894.

it is impossible to say whether the lacking fluid in one or the other experiment was entirely absorbed, or whether it was simply improperly introduced or unsatisfactorily removed. Again, Hamburger never mentions the size of the rabbit employed in his experiments. This, however, is quite an important point, since two animals of a different size might show a difference in the rapidity of absorption, as has indeed been claimed by G. Wegner.* But supposing that two rabbits of different sizes be found to show the same rate of absorption; this might be attributed to a difference in the results due to the ligation of the lymphatic ducts in one of the rabbits. Finally, the number of experiments which Hamburger reports are by far too small to permit such a sweeping conclusion as the exclusion of the lymphatics from taking part in the absorption of lymph. In fact, this author offers only one experiment for each of the fluids employed, and does not state whether or no he made others which are not included in his report. It may be worth while to quote all his experiments which have any bearing on our subject.

† A. Exp. 8.—Injected into a normal rabbit 150 cubic centimetres of a solution of sodium chloride (0.94 per cent); removed after half an hour 89 cubic centimetres; absorbed 61 cubic centimetres.

† B. Exp. 26.—Injected 150 cubic centimetres of the same solution into a rabbit whose innominate vein was ligated; removed after half an hour 93 cubic centimetres; absorbed 57 cubic centimetres.

A. Exp. 13.—Injected into a normal rabbit 150 cubic centimetres of concentrated horse serum; removed after two hours 140 cubic centimetres; absorbed 10 cubic centimetres.

B. Exp. 27.—Injected into a rabbit whose left innominate vein was ligated 150 cubic centimetres; removed after two hours 144 cubic centimetres; absorbed 6 cubic centimetres.

A. Exp. 14.—Injected into a normal rabbit 150 cubic centimetres of two-per-cent sodium chloride; removed after an hour 109 cubic centimetres; absorbed 41 cubic centimetres.

B. Exp. 28.—Injected into a rabbit whose left innominate vein was

* G. Wegner, *Archiv für klinische Chirurgie*, Bd. xx.

† The letters A and B are our additions to show the corresponding experiments for one and the same fluid which do not follow one another in Hamburger's article.

ligated 150 cubic centimetres of the same fluid; removed after an hour 133 cubic centimetres; absorbed 17 cubic centimetres.

A. Exp. 18.—Injected into a normal rabbit 150 cubic centimetres of rabbit serum, diluted with half as much water; removed after an hour 89 cubic centimetres; absorbed 61 cubic centimetres.

B. Exp. 29.—Injected into a rabbit whose innominate vein was ligated 180 cubic centimetres; removed after an hour 138 cubic centimetres; absorbed 42 cubic centimetres.

A. Exp. 20.—Injected into a normal rabbit 150 cubic centimetres of 0.5-per-cent sodium chloride; removed after half an hour 89 cubic centimetres; absorbed 61 cubic centimetres.

B. Exp. 30.—Injected 150 cubic centimetres of same fluid into a rabbit whose left innominate vein was ligated; removed after half an hour 85 cubic centimetres; absorbed 65 cubic centimetres.

We have here altogether five pairs of experiments, each pair dealing with a different kind of fluid. The one with the concentrated horse serum counts for little, as hardly any absorption was noted in either of the experiments. Of the remaining four parallel experiments, two show a decided diminishing effect of the ligation of the thoracic duct upon the absorption, while two show no such an effect. And yet these two parallel experiments are the only ones upon which Hamburger bases his categorical conclusions.

Our experiments which chronologically preceded those reported in the first part were also made on rabbits which were narcotized with chloral. The injections were made, just as in other experiments, by means of a syringe with a blunt point being introduced into the abdominal cavity through a small opening, which was then sutured and clamped. The fluid had a temperature of 99° F.; the rabbit remained on the holder during the entire time given for absorption, which was in all cases forty minutes. After killing the rabbit and opening the abdomen, the collecting of the remaining fluid was done very carefully, partly by means of a syringe or a pipette, and partly by means of absorbent cotton, which is well adapted for taking up every drop of fluid from all the corners and folds in the abdominal cavity. In the majority of the experiments a 0.75-per-cent solution of sodium chloride was employed, but 0.92-per-cent, 0.6-per-cent, and

0.3-per-cent solutions, respectively, were also tested, as will be seen in some of the protocols. It is unnecessary to recite the protocols of all our experiments or to report them chronologically as they were made. We shall only select certain examples from among them to report briefly, arranging them in such a manner as to bring out more clearly what can be learned and what can not be learned from this line of experimentation.

A series of experiments, accidentally following one another, seemed to demonstrate the importance of the lymphatics in the process of absorption. These experiments will be reported first:

Exp. 31.— Female rabbit, 1,960 grammes; injected into abdominal cavity 100 cubic centimetres of 0.75-per-cent sodium chloride; killed after forty minutes; collected from peritoneal cavity 65 cubic centimetres; absorbed 35 cubic centimetres.

Exp. 32.—Female rabbit, 2,100 grammes; right innominate vein ligated; * injected 100 cubic centimetres of 0.75-per-cent sodium chloride; killed after forty-five minutes; collected again 87 cubic centimetres; absorbed 13 cubic centimetres.

Exp. 33.—Female rabbit, 1,920 grammes; left innominate vein ligated; injected 100 cubic centimetres of same solution as before; killed after forty minutes; collected 81 cubic centimetres; absorbed 19 cubic centimetres.

Exp. 34.—Female rabbit, 2,400 grammes; right and left innominate vein ligated; injected into abdominal cavity 98 cubic centimetres of the same solution; killed after forty minutes; collected 86 cubic centimetres; absorbed 12 cubic centimetres.

This series of experiments seemed to demonstrate that the lymphatics exert quite a preponderating influence upon the removal of the fluids from the peritoneal cavity, the ligation of both innominate veins reducing the absorption by about 66 per cent. And it was rather surprising to find the right lymphatic duct taking such a prominent share in the absorption. Experiments giving results similar to the above were met with more than once and also under

* It is hardly necessary to state again that the ligations of the innominate veins were made for the purpose of preventing the lymphatic duct from emptying its contents into the circulation, as a direct ligation of the duct is hardly practicable in rabbits.

different conditions; we saw in parallel experiments a distinct influence of the ligation upon the absorption of fluids like 0.92-per-cent sodium chloride, the absorption of which is very slow, and also upon the absorption of 0.3-per-cent sodium chloride, which disappears quite quickly from the peritoneal cavity. It is obvious, however, that the small quantities observed to be absorbed after ligation would only warrant the claim of a diminution in the absorption due to the ligation, provided that it could first be shown that there is quite a stable coefficient for the absorption of fluid of a certain density and in a certain time from the abdominal cavity of normal animals. Hamburger seems to have presupposed such a stability without further proof, or else he would not have been satisfied with only one experiment for each sort of fluid. We have made quite a large number of observations on the absorption in normal animals. At first, indeed, it seemed that the absorbed quantities did not vary very much, and that the smallest quantity absorbed in normal rabbits was still distinctly larger than the quantities absorbed in those with ligated veins. For the 0.75-per-cent solution of sodium chloride, which we employed most frequently, 35 cubic centimetres seemed to be the average amount absorbed in forty minutes. We will illustrate this by two experiments:

Exp. 18.—Female rabbit, 1,520 grammes; starved for twenty hours; injected 98 cubic centimetres of 0.75-per-cent sodium chloride; killed after forty minutes; collected 61 cubic centimetres; absorbed 37 cubic centimetres.

Exp. 28.—Male rabbit, 2,400 grammes; not starved; injected 100 cubic centimetres of 0.75-per-cent sodium chloride into peritoneal cavity; killed after forty minutes; collected 65 cubic centimetres; absorbed 35 cubic centimetres.

Other experiments showed similar results. In the further course of our researches, however, we encountered results entirely at variance with the above. As will be seen in the following group of experiments, among our normal rabbits there were not only instances in which the absorbed quantities were not larger than those found in rabbits with ligated veins, but some animals from which we collected more than we had injected into their abdominal cavities:

Exp. 36.—Female rabbit, 1,700 grammes; injected 100 cubic centimetres of 0.75-per-cent sodium chloride; killed after forty minutes; collected 75 cubic centimetres; absorbed 25 cubic centimetres.

Exp. 30.—Female rabbit, 1,200 grammes; injected 100 cubic centimetres of 0.75-per-cent sodium chloride; killed after forty minutes; collected 80 cubic centimetres; absorbed 20 cubic centimetres.

Exp. 34.—Female rabbit, 1,800 grammes; injected 100 cubic centimetres; killed after forty minutes; recovered 82 cubic centimetres; absorbed 18 cubic centimetres.

Exp. 38A.—Male rabbit, 2,300 grammes; injected 100 cubic centimetres of 0.75-per-cent sodium chloride; killed after forty minutes; recovered 87 cubic centimetres; absorbed 13 cubic centimetres.

Exp. 40B.—Rabbit, 1,080 grammes; starved forty-eight hours; injected 100 cubic centimetres of 0.75-per-cent sodium chloride; killed after forty minutes; collected 90 cubic centimetres; absorbed ten cubic centimetres.

Exp. 39A.—Female rabbit, 2,400 grammes; injected 100 cubic centimetres of 0.75-per-cent sodium chloride; killed after forty minutes; recovered 104 cubic centimetres. No abnormal condition discoverable. Recovered 4 cubic centimetres more than injected.

The following pair of experiments, which were made exactly at the same time and under the same conditions, are very instructive:

Exp. 35A.—Female rabbit, 1,780 grammes; not starved; ligated right innominate vein; injected 100 cubic centimetres of 0.75-per-cent sodium chloride; killed after forty minutes; collected 93 cubic centimetres; absorbed only 7 cubic centimetres (influence of ligation?).

Exp. 35B.—Female rabbit, 1,800 grammes; injected 100 cubic centimetres of 0.75-per-cent sodium chloride; killed after forty minutes; recovered 112 cubic centimetres. Nothing abnormal; recovered 12 cubic centimetres more than injected.

For these remarkable differences in the amount of absorption during the same length of time and with the same kind of fluid we could make responsible neither the size nor the sex of the rabbit, nor the state of its digestion, nor, in fact, any other recognisable condition. It seemed simply that in rabbits each individual has its own degree of capability of absorption, depending upon certain, as yet unknown, conditions, which vary considerably in different individuals.

This condition was established not only for the saline of 0.75 per cent, but also for saline of a concentration of 0.92 per cent, which is, according to Hamburger, isotonic with the serum of the rabbit; and also for hypotonic salt solutions of the concentrations of 0.6 and of 0.3 per cent. The difference in the absorption between the isotonic and hypotonic fluids is striking enough to be recognisable even in the great variety of the absorbable quantities of each sort of fluid. This point is well illustrated by the two following experiments, which show in addition the interesting fact that an individual which absorbs less of one kind of fluid also absorbs less of another kind of fluid:

Exp. 51.—Rabbit, 960 grammes; starved twenty hours; injected 97 cubic centimetres of a 0.92-per-cent solution; after forty minutes rabbit put into deep ether narcosis; abdomen opened, all the fluid collected, recovered 75 cubic centimetres; absorbed, 22 cubic centimetres; the abdominal incision sutured carefully; injected 99 cubic centimetres of 0.3-per-cent sodium chloride; after forty minutes rabbit killed; recovered 30 cubic centimetres; absorbed 69 cubic centimetres.

Exp. 52.—Rabbit, 1,200 grammes; starved twenty-four hours; injected 99 cubic centimetres of 0.92-per-cent sodium chloride; etherized; after forty minutes abdomen opened and fluid collected; recovered 91 cubic centimetres; absorbed 8 cubic centimetres. Abdomen closed, injected 100 cubic centimetres of 0.3 per-cent sodium chloride; after forty minutes rabbit killed; collected 77 cubic centimetres; absorbed 22 cubic centimetres.

The first experiment shows the most ready absorption for the isotonic fluid (22 cubic centimetres), and also a good absorption for the hypotonic fluid (69 cubic centimetres). In the second experiment the isotonic fluid is poorly absorbed (9 cubic centimetres), and the hypotonic fluid is also comparatively scantily absorbed (23 cubic centimetres).

We shall append here a few more experiments showing the difference in the absorption between the isotonic and hypotonic fluids and the variability of the absorption for each kind of fluid:

Exp. 45.—Rabbit, 1,170 grammes; not starved; injected 99 cubic centimetres of 0.92-per-cent sodium chloride; killed after forty minutes; collected 85 cubic centimetres; absorbed 14 cubic centimetres.

Exp. 44.—Rabbit, 1,170 grammes; starved twenty-four hours; ligated right innominate vein; injected 97 cubic centimetres of 0.92-per-cent sodium chloride; after forty minutes collected 85 cubic centimetres; absorbed 12 cubic centimetres.

Exp. 46.—Rabbit, 1,440 grammes; starved twenty-four hours; ligated right and left innominate veins; injected 100 cubic centimetres of 0.92-per-cent salt solution; killed after forty minutes; collected 97 cubic centimetres; absorbed 7 cubic centimetres.

The largest quantity of the isotonic solution of sodium chloride which we have seen absorbed in forty minutes was 22 cubic centimetres. It is surprising to find that in the one experiment recorded by Hamburger with the isotonic solution of 0.94-per-cent sodium chloride, absorption during only thirty minutes was 61 cubic centimetres. And it is still more surprising to see that also for the hypotonic solution of 0.5-per-cent sodium chloride, Hamburger records the absorption of 61 cubic centimetres during thirty minutes. Our experiments have certainly convinced us that nothing in this entire question of absorption is so constant as the striking difference in the quantities absorbed according as the fluids are isotonic or hypotonic. This difference in the absorption can be already recognised unmistakably in fluids that present such a small difference in concentration as that between 0.92 per cent and 0.75 per cent. The quantity absorbed becomes markedly increased with the decrease in the concentration of the salt solutions, as is clearly demonstrated in Experiments 51 and 52 quoted above, and also in the following experiments:

Exp. 56.—Male rabbit, medium size; injected 95 cubic centimetres of 0.6-per-cent sodium chloride; killed after forty minutes; collected with absorbent cotton 45 cubic centimetres of a bloody fluid; absorbed 50 cubic centimetres.

The next two experiments, the protocols of which are given more in detail, seem to demonstrate quite positively that the lymphatics have no influence at least upon the absorption of hypotonic solutions of 0.6 per cent. This point, however, will be dwelt upon later on.

Exp. 57.—Rabbit, 1,440 grammes; thread around right innominate vein not tied; injected 100 cubic centimetres of 0.6-per-cent salt solution; etherization after forty minutes; abdomen reopened; fluid collected

by means of absorbent cotton held in forceps; fluid very bloody; collected 56 cubic centimetres; waste estimated 5 cubic centimetres; absorbed about 39 cubic centimetres. Ligature around right innominate vein tied; abdominal wound sewed up and clamped; injected again 93 cubic centimetres of 0.6-per-cent salt solution; reopened after forty minutes; fluid bloody; collected in the same manner as before 54 cubic centimetres; waste, about 4 cubic centimetres; absorbed about 35 cubic centimetres. Autopsy: The blood came from injury to liver; ligature included phrenic nerve.

Exp. 58.—Rabbit, 2,140 grammes; threads round both innominate veins not tied; injected 100 cubic centimetres of 0.6-per-cent salt solution; after forty minutes etherized; fluid collected with cotton, 40 cubic centimetres; waste, 8 cubic centimetres; absorbed about 52 cubic centimetres. Abdomen closed; right innominate vein ligated; injected 99 cubic centimetres of same fluid; abdomen reopened after forty minutes; after a few minutes of artificial respiration by tracheal cannula and bellows, collected with cotton 47 cubic centimetres; waste, 2 cubic centimetres; absorbed 50 cubic centimetres. Abdomen closed again; left innominate vein also ligated; injected 100 cubic centimetres of the same fluid; after forty minutes reopened; for a few minutes artificial respiration again employed; rabbit, however, alive; clear fluid collected 43 cubic centimetres; no waste; absorbed after ligation of both veins 57 cubic centimetres. Autopsy: Some fluid in pleural cavity; nothing abnormal otherwise.

It must be stated that for the salt solutions of 0.6 per cent there has been quite a remarkable constancy in the quantity absorbed during forty minutes; it varied only between 57 and 50 cubic centimetres, 39 cubic centimetres in Experiment 57 being the only exception, and in this case there was some blood oozing from the injured liver, so that it is impossible to say how much of the absorbed solution was compensated for by the blood.

The difference in the absorption between the solutions of 0.6 per cent and 0.3 per cent in our experiments was less striking than that existing between the solutions of 0.92 per cent and 0.75 per cent, or between the solutions of 0.75 per cent and 0.6 per cent. Parallel experiments have shown an apparent influence of the lymphatics upon the absorption of the 0.3-per-cent salt solution, as will be seen from the following experiments:

Exp. 41.—Female rabbit, 2,100 grammes; starved twenty hours; injected 100 cubic centimetres of 0.3-per-cent sodium chloride; killed after forty minutes; collected 41 cubic centimetres; absorbed 59 cubic centimetres.

Exp. 42.—Female rabbit, 1,620 grammes; starved twenty hours; right innominate vein ligated; injected 100 cubic centimetres of 0.3-per-cent salt solution; killed after forty minutes; collected 54 cubic centimetres; absorbed 46 cubic centimetres.

Exp. 43.— Female rabbit, 1,100 grammes; not starved; ligated right and left innominate veins; injected 100 cubic centimetres; killed after forty minutes; collected 67 cubic centimetres; absorbed 33 cubic centimetres.

The coincidence of the ligation of the lymphatics with the diminished absorption in the last two experiments, again would seem to indicate that the lymphatics influence the absorption. But it must be remembered that the absorption of the 0.3-per-cent salt solution in the normal rabbit shows also quite a variation in the quantities disappearing in forty minutes. The largest quantity we have noted was 69 cubic centimetres and the smallest 23 cubic centimetres.

Thus far we have pointed out the variability in the absorption of fluids in normal rabbits. We will now touch upon this point as it affects rabbits with ligated lymphatics. Here we have to speak mainly of our experience with the salt solution of 0.75 per cent, as the experiments made with fluids of other concentrations were not numerous enough to justify us in speaking of rules and exceptions. In rabbits in which either the right innominate vein alone or both the right and left veins were ligated we have noted without any exception an absorption of only comparatively small quantities—22 cubic centimetres being the largest and 7 cubic centimetres being the smallest quantity thus far noted. But with the left innominate vein alone ligated other results were observed. We find on record the two following experiments where the quantities absorbed were as large as in the normal animals:

Exp. 12.—Medium-sized rabbit, left innominate vein ligated; injected 95 cubic centimetres salt solution; rabbit breathed spontaneously, but apparently principally with right side; killed after forty minutes;

collected about 51 cubic centimetres; absorbed 44 cubic centimetres. Left phrenic included in ligature. This was the very first experiment in which we tried the ligation of the innominate veins.

Exp. 17.—Male rabbit, 1,635 grammes; tracheotomy; left innominate vein ligated; injected 98 cubic centimetres salt solution; artificial respiration; abdomen opened after forty minutes; collected 68 cubic centimetres; fluid somewhat bloody; absorbed 30 cubic centimetres. Experiment continued for ligation of vena cava inferior, etc.

This last experiment, however, can be excluded on account of the artificial respiration, which, as we often saw, is a great help to the absorption. But there remains the first experiment with the unusual absorption of 44 cubic centimetres, a quantity which we met in normal rabbits with a solution of 0.75 per cent only once, and this was in the experiment following closely the other one.

Exp. 13.—Medium-sized rabbit; injected 75 cubic centimetres salt solution; killed after forty minutes; collected 30 cubic centimetres; absorbed 45 cubic centimetres.

In both of these experiments, and only in these two, sponges were used for the collection of the fluid. It is possible that the sponges were not pressed out sufficiently.

In summing up the results which can possibly be derived from this line of experiments, our attention is engaged in the first place by the question of the participation of the lymphatics in the act of absorption of fluids from the abdominal cavity. In this respect we learn, in the first place, two negative points.

Although in one or two parallel experiments the rabbit with ligated thoracic duct may show as much absorption as the rabbit whose thoracic duct was not ligated, this does not justify the conclusion that the lymphatics do not aid perceptibly in the act of absorption, because the quantities absorbed at the same time in the normal rabbits vary considerably; and it is quite possible that the rabbit whose duct was ligated would, without ligation, have shown a much larger absorption. Hamburger's conclusion, based upon only two experiments, that the ligation of the right duct does not affect the absorption, is therefore assuredly not justified.

On the other hand, although a number of our experiments have shown us a diminished absorption in rabbits with ligated lymphatic ducts, we have also no right to deduce with absolute certainty that the ligation was the cause of the diminution in absorption, because the animal might by accident have been one of that class of rabbits which, even without ligation, would have shown the same poor absorption.

Our experiments (57 and 58) with the 0.6-per-cent salt solution, where we have seen in one and the same animal after ligation about the same quantities absorbed as before ligation, would certainly appear to be conclusive against any participation of the lymphatics in the process of absorption if the experiment could have been carried out without any flaw. This, however, was not the case. The deep etherization and the extensive handling of the peritonæum for the purpose of collecting all the fluid from the first injection affected the vitality of the rabbit to such a degree as to require some artificial respiration to keep the animal alive; and there can be no doubt that artificial respiration tends greatly to increase the absorption and might even overcompensate for the diminution caused by the ligation of the lymphatic ducts; and then there was always some waste, and the fluid was more or less bloody.

It may, however, be remarked incidentally that the fact that the absorbed quantities before and after ligation are the same does not necessarily mean that the paths of absorption must have been the same. As has been already stated in the first part, the ligation of the lymphatic duct raises the pressure within the lymph spaces, and thus facilitates the filtration of the lymph directly into the capillaries. It could, therefore, be claimed that absorption takes place before ligation by the route of the lymphatics and after ligation directly by filtration into the blood vessels, both modes of absorption being equally good, so that the absorbed quantities are in the end the same.

Thus far we have derived from our experiments only negative results—i. e., they have taught us that this line of experimenting can not furnish us with a decisive proof for either of the conflicting theories as to the mode of absorption from the peritoneal cavity. We

think, however, that our experiments have demonstrated a positive fact which gives at least some support in favour of the theory that the lymphatics are of essential value in the act of absorption.

If the absorbed quantities be arranged in tables, according to the density of the fluid experimented with (0.92, 0.75, 0.6, 0.3 per cent), it will be seen at a glance that for each fluid there is a considerable fraction among the number of experiments in which the quantities vary but little. Thus for the 0.75-per-cent salt solution, with which we experimented most often, we found in more than one half of our experiments that the quantity which disappears in forty minutes varied between 30 and 40 cubic centimetres, though the remainder of our experiments have shown a great variability, but only in a descending order, of the missing amounts of fluids. The same results were obtained also in our experiments with 0.92- and 0.3-per-cent solutions of sodium chloride. And it is by the character of the quantities in the majority of the experiments and also by their upper limit that we recognise the difference in the absorbability of fluids of different concentrations. (In the experiments with the 0.3-per-cent solution the largest absorbed quantity was 69 cubic centimetres; in the 0.75-per-cent, 45 cubic centimetres; and in the 0.92-per-cent, 22 cubic centimetres.)

Now, if we glance at the records of the quantities absorbed in the rabbits whose innominate veins were ligated, we notice that the table as a whole differs strikingly from that obtained from normal rabbits, though for both series of experiments one and the same fluid was used. In the large number of rabbits in which a solution of 0.75 per cent was injected and in which either both innominate veins were ligated or the right one alone, the largest quantity absorbed was 22 cubic centimetres. The same applies also to all cases in which the left innominate vein was ligated, except in our very first experiment, in which 44 cubic centimetres were absorbed.*

* In the latest number of Virchow's *Archiv* (Bd. cxliii, Heft 1) we find in an article by Maximilian Sulzer the statement (p. 104) that in a few cases he found a branch of the thoracic duct entering the innominate vein 0.5 centimetre below the entrance of the main duct. This branch was considerably dilated in cases in which the thoracic duct was ligated. Could not the

We do not think, however, that this single experiment, in which the methods employed in collecting the fluid were still imperfect, can change materially the impression to be derived from the entire table. The fact therefore remains that from a comparatively large number of experiments with ligated lymphatic ducts we obtain a table of absorbed quantities which is markedly on a lower scale than that derived from experiments with normal rabbits.

In other words, though we could not accept the single experiments with ligation as a positive proof in favour of the lymphatics, we believe that the bulk of our experiments show, at least with considerable probability, that the lymphatics exert a perceptible influence upon the act of absorption of fluids from the peritoneal cavity.

If the preceding conclusion is correct, it implies also another positive point of interest—viz., that the absorption is visibly impaired by the ligation of the right lymphatic duct. We had reason to arrive at a similar conclusion from a few of the experiments we made with potassium ferrocyanide.

Aside from the significance of the lymphatics for absorption there are a few other points bearing upon the question of absorption which are to be learned from our experiments and which deserve special mention.

We have already repeatedly referred to our observation that the quantities absorbed from the abdominal cavity during forty minutes increased with the decrease in the concentration of the fluid introduced. The difference in the absorption between salt solutions of 0.92 per cent and of 0.75 per cent is striking, and there is a still more marked difference between the results with the 0.75-per-cent solution and those of the 0.6-per-cent solution. The difference between the solutions of 0.6 per cent and 0.3 per cent is less pronounced in our experiments. This fact demonstrates the importance of the rôle played by osmosis in the absorption from the peritoneal cavity. In the few experiments of Hamburger * we do not find any difference in the

exceptional existence of such a branch explain the exceptional failure of the ligation of the left innominate vein, which was certainly applied above this branch?

* Hamburger, loc. cit.

absorption of solutions of 0.92-per-cent and of 0.5-per-cent sodium chloride.

In the experiments of Orlow,* however, the effect of concentration upon absorption was so easily recognizable that this author did not hesitate to draw the general inference that the absorption of the fluid increases in inverse ratio to its concentration.

Another point of interest is the variation in the absorption of fluids of the same concentration. Other authors before us have spoken of this inconstancy in absorption, and have laid great stress upon the proportion of the quantity of the introduced fluid to the weight of the body. We took almost constantly the same quantities for our injections, and, as far as our experiments upon rabbits go, the size of the body seemed to have very little influence upon the amount of absorption; we have seen small rabbits absorb quite large quantities and large rabbits absorb only quite small quantities. In fact, the variation in the absorption seemed to be entirely independent of the size, sex, stage of digestion, or any other condition accessible to our observation. And not only was the absorption sometimes exceedingly scanty, but in a few cases even more was collected than had been put in when no morbid or abnormal condition could be discovered which could be held responsible for this striking occurrence. As yet it is impossible to decide whether these wide variations in the absorption are due to some individuality in the rabbits—i. e., whether the same rabbit would always show the same degree of absorption, or whether they are due to unknown but transitory conditions in the animals—i. e., whether one and the same rabbit would at different times absorb different quantities.

Without attaching much importance to the fact, it may nevertheless be mentioned that all our animals which showed a scanty absorption were obtained from one source, while all those showing a higher absorption came from elsewhere.

*Orlow, loc. cit.

Some Experiments on Absorption from the Peritoneal Cavity of Dead Rabbits.

To Hamburger * belongs the credit of being the first to discover the remarkable fact that fluids disappear from the peritoneal cavity even in the case of dead animals. Now, if this disappearance is due to absorption—i. e., to the entrance of the fluid into the blood—its occurrence would seem to demonstrate that absorption can be accomplished without the assistance of the vital forces. Heidenhain,† however, who with Orlow believes in vital force for the act of absorption from the abdominal cavity, and who was naturally startled by the communication of Hamburger, claims in his latest article that absorption, according as it takes place in the living or in the dead body, is a radically different process, and he refers us to a future article for particulars on this point.

This question of absorption in the dead body is probably destined to excite general attention and to provoke much discussion. As yet there are on record only the few experiments published by Hamburger. In finding an explanation of the fact, it would seem that we should not encounter any difficulty provided only that we assume that absorption is carried on by the lymphatics, since, according to Heidenhain ‡ and others, the flow of lymph from the thoracic duct continues for a long time after death. Hamburger, however, denies the truth of such an assumption on the ground that the ligation of the thoracic duct does not influence absorption. But here again this author offers only one experiment with ligation of the thoracic duct.

We find in our protocols a number of observations on the absorption in dead rabbits, of which a few will be recorded here. The experiments were made exclusively with solutions of 0.75-per-cent sodium chloride, which had, of course, only room temperature.

Exp. 35.—Quoted above; 35 cubic centimetres absorbed in forty minutes during life. Abdomen sewed up; injected 100 cubic centimetres;

* Hamburger, loc. cit.

† Heidenhain, Pflüger's *Archiv*, Bd. lxii, p. 320.

‡ *Ibid.*, Bd. xlix.

opening clamped; collected after forty minutes 70 cubic centimetres; absorbed 30 cubic centimetres; tissue a little imbibed.

Exp. 18.—Quoted above. Absorbed during life 37 cubic centimetres. Abdomen sewed up; injected 100 cubic centimetres; after one hour and forty minutes collected 20 cubic centimetres; absorbed 80 cubic centimetres. Stomach and intestines much distended with gas.

Exp. 39.—Quoted above. While alive injected 100 cubic centimetres, and recovered after forty minutes 104 cubic centimetres. Abdomen sewed up; injected 100 cubic centimetres coloured with Prussian blue; after four hours and fifteen minutes recovered only 10 cubic centimetres; absorbed 90 cubic centimetres. Intestines and stomach exceedingly distended.

The absorption in these rabbits, which took place after death, was certainly not inferior to that which occurred during life. It was thought, however, that the marked distention of the intestines might have served as a mechanical factor to produce filtration. In the following experiments, therefore, the viscera were removed:

Exp. 29.—Rabbit, 2,030 grammes; injected 94 cubic centimetres; killed after forty-five minutes; collected 74 cubic centimetres; absorbed 20 cubic centimetres. Rabbit exceedingly fat. Stomach and intestines removed, abdominal cavity cleansed, sewed up; injected 80 cubic centimetres; after two hours and a half collected 64 cubic centimetres; absorbed 16 cubic centimetres.

Exp. 30.—Rabbit, 1,200 grammes; injected 100 cubic centimetres; killed after forty minutes; collected 80 cubic centimetres; absorbed 20 cubic centimetres. Stomach and intestines removed, cavity cleansed and sewed up; injected 80 cubic centimetres; after two hours and a half collected 60 cubic centimetres; absorbed 20 cubic centimetres; posterior wall of cavity œdematous.

From these and other of our experiments, it could be seen that with the removal of the viscera the absorption was considerably diminished. The fluid gathered on both sides of the spinal column without any pressure. It must be remembered, however, that the diminution of absorption might also be attributable to the removal of the visceral peritonæum.

The experiments with ligation of the lymphatic ducts did not give

uniform results. The following seemed to demonstrate that the lymphatics control the absorption from the abdominal cavity of the dead rabbit also:

Exp. 22.—Male rabbit, 1,080 grammes; right innominate vein ligated by a bowknot; rabbit killed; injected 97 cubic centimetres; collected after two hours and three quarters 89 cubic centimetres; absorbed 8 cubic centimetres. Knot around vein untied, abdomen closed by continuous suture; injected 94 cubic centimetres, some fluid escaping; overflow carefully collected, 47 cubic centimetres; waste liberally estimated 10 cubic centimetres. Abdomen reopened after two hours and three quarters; collected 7 cubic centimetres; estimated absorption, 30 cubic centimetres. Abdomen closed again carefully by interrupted suture; injected 64 cubic centimetres. No escape; waste estimated 7 cubic centimetres. Reopened after two hours and three quarters; collected 7 cubic centimetres. Absorption, 50 cubic centimetres; intestines much distended.

In some other experiments, however, the ligation did not seem to exert any influence upon the absorption.

Exp. 32.—Quoted above. Right innominate vein ligated; absorption in forty minutes while alive only 13 cubic centimetres. Abdomen of dead rabbit carefully sutured; injected 98 cubic centimetres; no escape and no waste of fluid; reopened; collected after forty minutes 65 cubic centimetres; absorbed 33 cubic centimetres. Tissue of abdominal cavity, especially of posterior wall, exceedingly imbibed.

Exp. 34.—Quoted above. Both innominate veins ligated; absorbed in forty minutes while alive 12 cubic centimetres. Abdomen sutured; injected 100 cubic centimetres; no fluid escaped; reopened after forty minutes; collected 73 cubic centimetres; absorbed 27 cubic centimetres.

In these and other experiments we have seen that when the veins were ligated there was more absorption during the same period in the dead than in the living body.

Our experiments on absorption in dead rabbits do not permit of a full comparison with this process as it occurs in the living animals, since in only a few of the former was the fluid removed after forty minutes, the time which was uniformly maintained in our experiments on the living. In these few experiments there was hardly any difference in favour of the dead, except in the animals with ligated

veins. In the experiments in which we removed the fluid after hours there was certainly a marked absorption. Even in Experiment 39, in which in forty minutes during life there was an increase of 4 cubic centimetres in the amount of the fluid, we find after death an absorption of 90 cubic centimetres in four hours and a half. But in all cases in which the fluid remained for hours the intestines were exceedingly distended with gas, thereby exerting a considerable pressure upon the fluid within the peritoneal cavity, and thus affecting the absorption like a strong constant massage. In the experiments in which the viscera were removed there was indeed constantly a strikingly diminished absorption, but of course this could be ascribed to the diminished pressure as well as to the removal of the visceral peritonæum with its absorbing lymphatics or blood capillaries.

The ligation of the innominate veins has not shown, at least in the majority of the experiments, any constant influence upon the absorption; on the contrary, in one and the same rabbit with ligated veins there was noted a good deal less absorption during forty minutes during life than after death, when the absorption seemed to be the same as in a normal rabbit.

It is a noteworthy fact that in our protocols of the experiments upon the living animals we find only now and then the remark that the tissue was somewhat imbibed.

In the protocols of the observations on the dead rabbits, however, we often find the remark that the tissue was exceedingly imbibed and œdematous, especially the posterior wall.

Discussion of the Results obtained.

Before attempting an interpretation of the results obtained from our experiments we shall recapitulate briefly the problem and the solutions which have been suggested by some other authors.

The fact was observed that fluid disappeared from the abdominal cavity. This disappearance having without any discussion been assumed to be identical with absorption into the blood, it remained only to decide whether the fluid entered into the blood by the way of the lymphatics or directly through the walls of the blood vessels. As,

according to one statement, the flow of lymph from the thoracic duct did not increase during this absorption, and as, according to another statement, the ligation of the thoracic duct did not influence the absorption, the conclusion was drawn by these authors that the fluid enters into the blood directly through the walls of the blood vessels.

But there is, as it would seem, an important gap in this reasoning. In the first place, nobody has proved or even attempted to prove that the fluid really enters the blood; and all that can be said for certain is that the fluid disappears from the peritoneal cavity. In the second place, the fluid within the peritoneal cavity can never enter directly into the blood vessels, as these are not directly exposed within the peritoneal cavity, but are embedded within the lymph spaces which are separated from the serous cavity by an epithelial membrane, just as the blood within the capillaries is separated from the lymph space by an endothelial membrane. The first question which confronts us when we see fluid disappearing from the abdominal cavity is: By what force does this fluid enter into the lymph spaces? And the next problem is not how does the new fluid within the lymph spaces enter the blood vessels, but rather, does it enter the blood? It is certainly conceivable that the fluid remains within the lymph spaces, and that its only effect upon the blood consists in the prevention of the usual transudation from the capillaries into the lymph spaces. Before these questions have been satisfactorily solved, the statement that the fluid does not go through the lymphatics, and consequently penetrates directly the walls of the blood vessels, must be considered as premature.

The origin of this oversight is to be found, as we believe, in the general practice of confounding the lymph spaces with the lymphatics, although every one, whatever his theories about the function of the lymphatics may be, will admit that the function of the lymph spaces is entirely different from that of the lymphatics, and certainly every one will concede, as a matter of course, that the structure of the lymph spaces is just as different from that of the lymphatics as from that of the blood vessels; nevertheless we do not know of any writer who assigns to the system of the lymph spaces a position independent of and separate from the lymphatics. Again and again

we find it stated in the literature that the lymph spaces are merely the roots of the lymphatics, which again many consider only as a branch current of the circulation. According to this view there are then at the disposal of any fluid in the body only two systems: the blood vessels and the lymphatics. Therefore if it is assumed as a proved fact that the fluid within the abdominal cavity does not enter the lymphatics, then the conclusion that it enters the blood vessels directly is inevitable. Furthermore, a study of the recent literature on the relations between blood pressure, lymph flow, and lymph formation has shown to us that the conclusions drawn from these experiments could only have been arrived at by underrating the importance of the lymph spaces as an independent system. It is not our purpose to enter into any particulars on the subject of lymph formation, but we mention these facts only as a further excuse for dwelling here at some length on our conception of the lymph spaces as an independent system and as an intermediate territory between the blood vessels and the lymphatics. We do not propose to offer any new experiments or histological observations, but shall take, on the contrary, the old well-known facts as a basis for our point of view against which, as we hope, the only objection which can be urged is that it is not new.

The so-called lymph spaces are those gaps, clefts, chinks, and interstices of all kinds naturally present between the elements or the groups of elements of the tissues of the entire body. They are of variable shape and size. The fine capillary space between two muscular fibres is certainly much smaller than the space between two muscle bundles, and the spaces in loose connective tissue are certainly much larger than those present in dense tissue, such as, for instance, is found in ligaments. Some of the spaces are lined with a few endothelial cells, but these are never sufficiently numerous to separate one space from another, though the freedom of communication varies considerably between the spaces of the same organ, and probably still more so between those of different organs. Certain strata of connective tissue extending over large areas of the body present convenient paths, connecting the parenchymatous spaces of several

organs. We may thus assume that all these spaces are more or less completely connected throughout the entire body and present a unity, and, as it were, a system of canalization. Throughout the course of this system and at all times are present variable quantities of fluid containing the nutrient material for the tissues as well as their waste products. In loose tissue the accumulation of fluid can be enormous. In fact, the size of lymph spaces seems to depend more upon the quantity of fluid present than upon structural limitations. The fluid spreads with more or less ease from one lymph space to another, the propelling forces being diffusion and mechanical pressure. The latter is caused, first, by contracting the muscles surrounding the lymph spaces, and, secondly, by gravity and external mechanical pressure (massage). Mechanical pressure is perhaps also produced by the chemical activity within the tissue, just as we see the chemical activity in the salivary glands producing a pressure which exceeds that found within the carotid arteries.

In these lymph spaces are embedded the capillaries of the blood vessels as well as of the lymphatics. Though the larger tubes of both kinds of vessels are well separated from their surroundings by membranous walls, the capillaries are separated from the lymph spaces only by a thin endothelial layer, and even this may possibly be provided with stomata, so that they are all well adapted to give up fluid to or receive it from the lymph spaces. In only a few exceptional cases does the fluid from the blood capillaries enter directly into the lymphatics, as happens in the case of the perivascular lymphatics of the central nervous system. As a rule, the fluid on leaving the blood enters the lymph spaces, and thence passes to the lymph capillaries; it never enters directly from the blood capillaries into the lymph capillaries. There is always some space between both kinds of capillaries, and they are said to be sometimes separated even by more than one lymph space.

Both systems of fluid-carrying vessels, blood vessels and lymphatics, possess in common many features which distinguish them sharply from the system of the lymph spaces. Both have the tubular character, the abundance of muscular and elastic fibres in the mem-

branes, the nervous control, the more or less limited capacity, the free and unembarrassed movement of the fluid, the possibility of free communication between all sections, and the unipolar direction of the constantly moving fluid. The arrangement of the three systems is suggestive of a tract of marshy land in a swamp separating two rivers, the larger one of which as it passes by sends out only fine arms into the swamp, while the smaller one, which originates there, collects its water from the marshy land to bring it back after a short course into the main river.

An important distinguishing feature is the difference in function. The real process of nutrition goes on within the so-called lymph spaces, but not in the blood vessels or in the lymphatics, which only serve as routes for transportation. A comparison with different portions of the alimentary tract will bring out these points more distinctly. The system of the lymph spaces may be compared to the stomach and the intestines, where the chief object of alimentation—digestion—is going on; while the blood vessels are to be compared with the œsophagus, the road for the food delivery; the lymphatics, finally, may thus be considered analogous to the rectum, the canal for the removal of the waste products.

There are certainly abundant reasons for considering the lymph spaces as an independent system for itself, and not as an appendix to either class of tubulated vessels. The reason for the general usage of classing the lymph spaces with the lymphatics is probably to be found in the fact that the fluids of the lymph spaces as well as those of the lymphatics have two striking features in common, their colourless appearance and their poverty in corpuscular elements, which are in striking contrast to the red colour and abundance of corpuscles in the blood. Such a classification, however, implies also the sweeping and unfounded assumption that both fluids not only present a similar outward appearance, but are actually of one and the same character, and that it is simply the fluid of the lymph spaces which is found flowing through the lymphatics and the thoracic duct. In fact all the analyses of "lymph" were made from the fluid coming from the lymphatic ducts or from the large lymphatic vessels, and it has been tacitly as-

sumed, and entirely without proof, that this lymph and the fluid within the tissue are one and the same in character.

We have no experiments to offer showing that the fluids are different or the nature of the difference if such exists. But, even as it is, there are good reasons against the supposition that the lymph in the thoracic duct is the unaltered fluid from the tissues. This latter is surely an admixture of three different elements: (1) The fluid transuding from the blood into the lymph spaces; (2) the same fluid minus the material taken out for the anabolism of the tissue; and (3) the products furnished by the catabolism of the tissue. Now the organs and tissues of different structures possessing different functions require a different material for their nutrition, and give up different waste products. The natural conclusion, then, must be that the fluid within the lymph spaces varies considerably according to the particular tissue and organ concerned. In contrast to these specific fluids of the tissues, the thoracic duct carries an admixture of all these fluids together, and also the fluid taken up from the intestinal canal, which even in a state of inanition is never quite empty.

It is, then, quite evident that the fluid coming from the thoracic duct can not be taken as representative of the fluid within the tissues even if it were admitted that the tissue fluid entered into the lymph capillaries as through an open gate without any change of character—an assumption, however, which is in the highest degree improbable, since the fluids in the lymph spaces are separated from the lumen of the capillaries by an endothelial membrane through which they can permeate only with the aid of some physical or "vital" force.

The identification of lymph with the tissue fluid leads to another error. In measuring the quantity of lymph coming from the thoracic duct, some writers think they determine with it also the quantity of fluid passing through the tissues. This would be equivalent to saying that the whole amount of fluid which transudes from the blood into the tissue, and which is there called lymph, leaves the tissue by way of the lymphatics. That this is a mistake is evident from the fact that large quantities of the tissue fluid pass out again by way of the glandular secretions without the intervention of the lymphatics

at all. It must be remembered that the secretion of saliva alone sometimes amounts to as much as two litres in twenty-four hours, not to mention all the other secretions and "juices" of the alimentary canal. Then again mucus, sweat, tears, milk, and other fluids must all be considered. The amount of milk alone secreted in twenty-four hours probably at times does not fall short by much of the amount of lymph coming from the thoracic duct, and in any case all the secretions taken together form a considerable quantity which is possibly larger than the whole amount of lymph flowing through the ducts. Now, since all these secretions derive their fluid directly from the lymph in the lymph spaces, it is impossible that the flow from the thoracic duct should give us any exact information as to the consumption of this lymph.

Again there are two other factors, though of undetermined character, of which the flow of lymph from the ducts can not give us any account. In the first place, the amount of fluid which is always present in the interstices of the tissue can vary within certain limits, the difference nevertheless being imperceptible or certainly not measurable. The semisolid character of most of the tissue is based not only upon the fluid within its elements—for instance, within the sarcolemma or neurolemma—but also upon the constant presence of fluid between them. The observation made by v. Brasol * and by Leathes † of the rapid entrance of large quantities of fluid into the blood following the injection of solutions of sugar or salt into the blood, while the flow of lymph from the thoracic duct is also increased (Heidenhain ‡), shows the presence of such labile fluid in the tissue. The second unknown quantity is the exact amount of fluid which at certain times and in certain places is undoubtedly returned directly back into the blood vessels.

In referring to this subject it may not be out of place to note the apparently contradictory position of Heidenhain. In his first article # on the subject of lymph formation he says that it is inconceivable that

* v. Brasol, Du Bois-Reymond's *Archiv für Physiologie*, 1884, p. 211.
† Leathes, *Journal of Physiology*, vol. xix, p. 1.
‡ Heidenhain, Pflüger's *Archiv*, Bd. xlix, p. 219.
# Pflüger's *Archiv*, Bd. xlix, p. 219.

under normal circumstances a part of the lymph should be returned directly to the blood, whereas in his last article * he says that it is his conviction that the blood capillaries are the essential paths for absorption from the peritoneal cavity. Whether Heidenhain has given up his first position or makes a distinction between the absorption of lymph and the absorption of other fluids from the peritoneal cavity, it is impossible to understand.

We thus see that the lymph coming from the lymphatic ducts represents neither in quantity nor in quality the fluid which is contained in the lymph spaces.

We shall therefore, to avoid further misconceptions, discard the terms "lymph spaces" and "lymph" for the spaces within the tissue and the fluid therein, and apply to them instead the commonly employed terms "interstitial spaces" and "tissue fluid," which are more appropriate, as they do not carry with them any hypothetical meaning.

Recapitulating our statement briefly, our position is as follows: The tissues of the body are permeated by a system of interstitial spaces which contain the tissue fluid. This system is, by virtue of its structure, its function, and its contents, an independent apparatus by itself, and not an integral part of either of the two systems of vessels, and the fluid within this system is likewise in many important respects different from the fluid of the blood vessels as well as from that of the lymphatics. The system receives fluid from the blood vessels which is applied in different organs and in different tissues in a specific way. Aside from its use for metabolic purposes for the tissues within the body, a part of the fluid is given up to the glands as material for their specific secretions, a part conveyed into the lymphatics, while possibly, under certain circumstances, a part is returned directly into the blood vessels. This process of transudation and distribution of the fluid is in the first place controlled by the physical forces of filtration and osmosis. Whether these are sufficient is just now a matter of discussion. We must say, however, that as yet we have not sufficient knowledge of the actual process, nor do we know enough of the laws of osmosis, especially in their application to living tissue, to justify

* Pflüger's *Archiv*, Bd. lxii, p. 330.

the establishment of such a far-reaching conception as a " vital force." For transformation of the tissue fluid into the glandular secretions, there are indeed a number of facts which justify the assumption that the glandular cells influence the process in a manner not explainable by our present knowledge of physical laws. The same can not be claimed, or at least not to the same extent, when we come to discuss the process of transudation of the fluid of the blood into the interstitial spaces. We are not going to criticise here the reasons which have led to the hypothesis that the capillary endothelia are endowed with a secreting capacity. We wish only to call to mind that in the experiments upon this question " interstitial spaces " and lymphatics, as well as " tissue fluid " and lymph, have not been kept apart. With regard to the mechanism of the entrance of fluid from the interstitial spaces into the lymphatics little can be said, as this subject has been studied least of all. That filtration may have an influence seems to be shown by the fact that the lymphatics can be injected simply by injecting the tissue. It is probable that osmosis also has something to do with the passage of fluid from the interstitial spaces through the endothelial membrane of the lymph capillaries. However, if we assign to the lymphatics the task of carrying off the waste products from the tissue fluid, a theory favoured somewhat by the absence of oxygen and the presence of carbonic-acid gas, there will be need of a more complicated mechanism to accomplish this selection of materials. For reasons to be stated later we are indeed inclined to favour the theory that the lymphatics drain off from the system of interstitial spaces such substances as are in their present state either foreign or useless or harmful to the tissue, and deposit them in the lymphatic glands, or carry them into the blood to be rebuilt there or to be excreted.

Returning now to our problem in hand, we repeat that the fact of the disappearance of fluid from the peritoneal cavity suggests as the very first point that the fluid entered into the interstitial spaces, and the first question to be answered is, By which means was this accomplished?

There are three possible explanations. The fluid of the peritoneal cavity can enter into the surrounding interstitial spaces: (1) Through

the stomata, which are claimed by some authors to exist between the epithelia of the peritoneal membrane; (2) by filtration; (3) by osmosis. With reference to the stomata, it is to be remarked that the latest writers, Kolossow * and Muscatello,† deny their existence, so that it may be best not to enter into a discussion of their availability for the purpose in question.

As for filtration, this process would presuppose that the pressure in the peritoneal cavity is higher than in the interstitial spaces, and we have no data which enable us to make a positive statement with regard to this point. We can offer, however, the following considerations: In a state of absolute rest the fluid in the peritoneal cavity of the living body stands probably under atmospheric pressure, while the fluid in the interstitial spaces is influenced by the pressure within the blood capillaries, which is probably somewhat higher than the atmospheric pressure. But the living body is very rarely at rest and the abdominal cavity more especially is nearly constantly under the influence of muscular contraction during inspiration as well as during expiration, which, in rabbits at least, is always active. In the dead body, again, the rapid development of gases within the intestines forms a new factor which exerts quite a considerable pressure upon the contents of the abdominal cavity, while, on the other hand, there is no increased elevation of pressure in the interstitial spaces due to the elevated blood pressure. We might then presume that there is in the living animal, as well as in the dead body, enough surplus pressure to bring some of the fluid of the abdominal cavity by filtration into the interstitial spaces. In support of this hypothesis we can quote a few facts which show the favourable influence of mechanical pressure upon the disappearance of fluid from the abdominal cavity. In experiments which we have not quoted we saw that massage of the abdomen, the application of a light bandage around the abdomen, or even the keeping of the rabbit upon its stomach increased the quantity of the missing fluid. All these facts, however, show only that a certain degree of mechanical pressure can accomplish an increase in the disappearance of fluid from the abdominal cavity; but there is noth-

* Kolossow, *Archiv für mikroskopische Anatomie*, 1894.

† Muscatello, Virchow's *Archiv*, Bd. cxlii, p. 327.

ing to show to exactly what extent the disappearance in our case was actually due to simple pressure—i. e., to filtration.

It is different with regard to osmosis. Here we have the positive proof that the fluid disappeared in inverse proportion to its osmotic pressure; the less concentrated the solution of sodium chloride injected, the more of it disappeared during the same period. The highest quantity which disappeared from the solution of 0.92 per cent was 22 cubic centimetres; the highest quantity from the solution of 0.75 per cent was 45 cubic centimetres; and from the 0.6 per cent, 69 cubic centimetres. Similar observations were made by Orlow,* in whose protocols we also find that hypertonic solutions were not absorbed as such, the solutions within the abdomen having first to become at least isotonic before any absorption took place.

We see also, in Orlow's protocols, that when isotonic fluids are introduced the remaining fluid within the abdomen has invariably a hypotonic pressure. And it has not as yet been investigated whether the isotonic fluid does not turn in the first few minutes hypotonic before the absorption takes place. We may thus state that the entrance of hypotonic fluid from the abdominal cavity into the interstitial spaces occurs mainly by osmotic pressure, perhaps assisted somewhat by filtration. The isotonic fluid, which is absorbed poorly, either first turns slightly hypotonic and is also absorbed by osmosis, or what little absorption occurs is caused entirely by filtration.

As the process of absorption goes on also through dead and prepared peritoneal membranes, we may assume that the fluid in the peritoneal cavity in dead animals enters into the interstitial spaces in the first place by osmosis, and in the second place by mechanical pressure (filtration) exerted upon the fluid by the rapidly developing gases within the intestines; but it is also possible that in the dead body osmosis has a smaller and filtration a larger share in the removal of the fluid in the living body.

With regard to the application of the laws of osmosis to our special problem, and to the process of absorption in the animal body in general, a few remarks may be added. The osmotic pressure of 0.92-per-

* Orlow, loc. cit.

cent sodium chloride, which is now, following the lead of Hamburger, generally taken as being equal to the osmotic pressure of the body fluid, has been established only for the blood serum. For the lymph flowing from the thoracic duct Leathes * found a somewhat higher degree, and for the lymph flowing from the cervical duct in horses Hamburger † records a much higher osmotic pressure than in the blood serum. For the tissue fluid within the interstitial spaces we have no knowledge whatsoever about the osmotic pressure. With reference to this point, it may be insisted again that fluid of any osmotic pressure brought into the abdominal cavity attains earlier or later invariably a certain uniform osmotic pressure. But the two statements which we have on this point differ as to what this pressure amounts to. While Hamburger, who employs the red-blood-corpuscle method, states that the fluid invariably attains the same osmotic pressure as the blood serum, we find in all the protocols of Orlow, who simply made chemical analyses of the fluid, that the solutions in the abdomen were constantly within the limit of 0.75- and 0.73-per-cent sodium chloride. This would mean that they become hypotonic with regard to the osmotic pressure of the blood serum. This is important when we remember that the fluid in the abdominal cavity is influenced in the first place by the tissue fluid.

Above all, however, we have to remember that we are handling osmotic laws as they are seen in final osmotic pressures and applying them to the process in the living body, where we have principally relations between initial or unsettled rates of osmosis. In this connection we would refer to a recent very instructive article by Lazarus-Barlow. ‡ He studied the ratio between the initial rates of osmosis of glucose, sodium chloride, and urea and their final osmotic pressure. He reaches the conclusion (p. 165) "that it is impossible to state from a determination of their freezing points that one solution is hypertonic, isotonic, or hypotonic as regards another solution of a different composition at pressures within the limits possible in the animal

* Leathes, loc. cit.

† Hamburger, Ziegler's *Beiträge zur pathol. Anatomie*, Bd. xiv.

‡ Lazarus-Barlow, *Journal of Physiology*, vol. xix, p. 140.

body." From his results (p. 160) "follows the paradox that if so-called isotonic solutions be placed on the two sides of a peritoneal membrane under conditions such as those we are now considering, osmosis can nevertheless occur. One may go even further and say that osmotic flow may take place from a solution having a higher final osmotic pressure toward a fluid having a lower final pressure."

The results were different when the equimolecular solutions were watery, faintly albuminous, or highly albuminous; and the results were again different when the separating membrane was copper ferrocyanide or prepared peritonæum. And we can reasonably assume that the difference is probably still greater when the separating membrane is unprepared peritonæum in the living body. We need therefore not be in despair if we can not explain all the particulars of the phenomenon under consideration by our present meagre knowledge of osmosis in its working in the complex condition of the animal body. Above all, we have certainly no right to appeal to "vital" force as long as we know so little of the working of the physical force we attempt to apply.

Our answer to the first question, therefore, is that the fluid which we have seen disappearing from the peritoneal cavity in the living as well as in the dead body entered the interstitial spaces by the process of osmosis assisted by filtration. The next question in order is: What becomes of this fluid within the interstitial spaces? The natural answer is that the fate of this fluid is probably the same as that of any other fluid present in excess within the interstitial spaces. Any accumulation of fluid in any region of the system of interstitial spaces has three ways open by which it can escape: (1) It can spread within the system of interstitial spaces into the neighbouring regions, either not affecting the blood and lymph vessels at all or affecting the blood vessels sufficiently to reduce the transudation into this region; (2) it can enter into the blood vessels through the walls of the capillaries or the veins; and (3) it can enter the lymphatics through the walls of the lymph capillaries.

When considering the further spreading within the neighbouring interstitial spaces, we must remember that the abdominal cavity

is surrounded with strata of muscular and connective tissues which are very appropriate for this purpose. The working forces for this spreading are, as we have pointed out above, diffusion and mechanical pressure, the latter being rendered quite considerable in the region in question by the respiratory motions and other factors. We should also remember that the entrance of fluid into the interstitial spaces occurs mainly by osmosis, which works well also against mechanical pressure. Now if a comparatively large quantity of a hypotonic solution enters rapidly into adjacent interstitial spaces, it can produce there quite a high mechanical pressure which might even exceed the pressure within the blood vessels, and certainly within the lymph vessels. This fluid, then, would not only suppress the transudation from the blood vessels, but it would enter also into the blood vessels by filtration, even against osmotic pressure. The same reasoning, of course, applies also to the lymphatics. Our answer to the second question raised above would then be, *a priori*, that the fluid which entered from the peritoneal cavity into the adjacent interstitial spaces partly spreads within the system of interstitial spaces from the neighbouring to the more distant tissues, partly enters the lymphatics, and partly invades also the blood vessels. If it is proved by experiment, as is indeed claimed by Heidenhain and Orlow, that during such a disappearance of fluid from the abdominal cavity the lymph flow from the thoracic duct is not increased, and if, further, we accept this as a proof that the disappeared fluid did not enter the lymphatics, our answer would have to be modified only with regard to the lymphatics, and we should say that the fluid spreads partly into the interstitial spaces and partly enters into the blood vessels. The conclusion which Heidenhain and Orlow have formed, that the whole of the fluid which has disappeared has entered directly into the blood vessels, is not warranted by the results of their experiments. That the fluid does not enter into the lymphatics is no proof that it enters into the blood vessels, so that their conclusion is, in fact, only a mere hypothesis. While it is certain that the disappeared fluid entered into the interstitial spaces, there is no proof whatever that it leaves the tissue spaces to enter into the blood vessels, a course which is the more open to

doubt since we see that filtration and osmosis are ineffective in bringing the fluid into the lymph capillaries. However, we think that the blood vessels possess an advantage over the lymphatics in their stronger current, which carries away every trespassing molecule to make room for another one.

As to the strength of the conclusion from the experiments of Heidenhain and Orlow with regard to the lymphatics themselves, we would only say here that the thoracic duct is not the only canal which carries the lymph from the abdominal cavity. In our experiment with larger quantities of salt solutions, as well as with the potassium ferrocyanide, we saw that the right lymphatic duct exerts also a decided influence upon the absorption from the abdominal cavity. In the experiments of Heidenhain and Orlow it is possible that the right lymphatic duct vicariously assumed the task of removing the share of the surplus allotted to the lymphatics, inasmuch as the lymph flowing from the thoracic duct through a cannula under atmospheric pressure is less equal to its task, as it is deprived of a helpful factor, the negative pressure which is supplied by the suction occurring in the jugular veins during each inspiration.

Our own experiments have shown that the ligation of the lymphatic duct certainly reduces the quantity of fluid that disappears from the abdominal cavity in a given period of time and to a noticeable degree. While insisting, on the one hand, that the experiments of Heidenhain and Orlow do not prove absolutely that the lymphatics do not carry off any of the fluid, we admit, on the other hand, that our experiments do not necessarily mean that the lymphatics carry off some share of the fluid. The influence of the ligation can be interpreted also by the assumption that the prevention of the removal of the usual quantity of lymph from the interstitial spaces causes there so many changes in the mechanical and osmotic pressures that only a smaller part of the normal quantity can be taken up by the interstitial spaces from the abdominal cavity. Thus the positive conclusions which we can draw from our experiments with the salt solutions must be restricted to the bare statement that the ligation of the lymphatics exerts a restricting influence upon the quantity of

fluid disappearing from the abdominal cavity, but the experiments do not indicate whether or not the lymphatics carry away a part of this fluid.

It is different, however, with our experiments made with strychnine and potassium ferrocyanide. Here we have seen distinctly that when the lymph ducts were open the effect of the strychnine upon the nervous system and the excretion of potassium ferrocyanide by the kidneys occurred much earlier than when the lymphatic ducts were ligated. This shows unmistakably that in the normal state strychnine and potassium ferrocyanide leave the interstitial spaces mainly, and perhaps exclusively, by the way of the lymphatics. When the lymph ducts are ligated the substances linger for some time within the interstitial spaces until the mechanical or osmotic pressure, or rather both, reach a condition which enables these foreign substances to enter directly into the blood vessels.

It was our experience with strychnine and potassium ferrocyanide which made us inclined, as stated above, to support the hypothesis that to the lymphatics, among other duties, is assigned the special task of carrying off from the interstitial spaces such products as are either foreign, useless, or harmful to the tissue, and to deposit them in the lymphatic glands or to convey them to the blood, to be rebuilt there or to be excreted.

Our position with regard to the lymphatics can be briefly defined as follows: Our experiments prove that strychnine and potassium ferrocyanide are carried mainly or exclusively by the lymphatics. It is further quite certain that ligation of the lymph duct on the one hand restricts the entrance of fluid from the abdominal cavity into the interstitial spaces, and on the other hand even assists foreign substances, like strychnine and potassium ferrocyanide, present in the interstitial spaces, in finally entering into the blood through the walls of the blood vessels. Of the hypotonic and isotonic solutions of sodium chloride introduced into the abdominal cavity, we merely know for certain that they enter with more or less ease into the interstitial spaces; of their final fate we can only state hypothetically that they partly enter also into the lymphatics, selecting those ducts which are

open and offer the best facilities for their transference into the circulation.

As to the absorption of fluid from the peritoneal cavities of dead animals, we have to admit that even without the vital force as an active factor in the absorption there are indisputable changes in the dead bodies which undoubtedly alter in some respects the mechanism of absorption in them. Though we know very little of the working of osmosis in animal bodies, we know enough to enable us to recognise the fact that in the living body we have to deal nearly always with relations between more or less initial rates of osmosis, while in the dead bodies we deal with conditions approaching more the final rates of osmotic pressure. There is no transudation of fluid from the blood vessels into the interstitial spaces, and there are no anabolic processes going on within the tissue. Furthermore, there are no longer muscular contractions to produce mechanical pressure for filtration, and there is no current either within the blood vessels or lymphatics. With respect to this last statement, it is true that the lymph flows from a cannula in the thoracic duct for some time after death; but this does not show that the lymph is moving through the thoracic duct when it is normally connected with the jugular vein any more than the flow of blood from a cut would prove that the blood is flowing within the uncut vein. The fact that there is no current in the innominate veins proves our assumption that the thoracic duct does not discharge its contents into the jugular vein.

These changes, however, apparently influence but very little the entrance of the fluid from the peritoneal cavity into the interstitial spaces. The laws of the final osmotic pressure permit the absorption by osmosis of hypotonic fluid, which was exclusively employed in our experiments with dead animals. Besides, the rapid development of gases within the stomach and intestines of dead animals forms a new and efficient factor for filtration. Thus we may say, as already stated above, that the entrance of the fluid into the lymphatic spaces occurs in dead animals also by osmosis assisted by filtration, and that, though filtration may have a larger and osmosis a smaller share in the process here than in a similar process in the living body, the final effect is

about the same. That the ligation of the lymphatic duct has no influence upon the quantity of fluid which disappears from the peritoneal cavity is natural enough, for even without ligation there is no current of lymph within the lymphatics of the dead body.

It must be different with the further disposal of fluid within the interstitial spaces of the dead animals. None, or almost none, of the fluid will enter either the blood or the lymph capillaries. Even if there should be enough of osmotic and mechanical pressure to make some molecules pass through into the lumen of the vessels, there is no current to carry off these molecules to make room for others, and the effect of simple diffusion within these minute vessels is exceedingly small. The fluid which has entered the interstitial spaces must, therefore, remain there, but in this case again there are no respiratory movements or other muscular contractions to effect a wider distribution over a larger area and through the entire thickness of the tissues. The fluid, therefore, remains in a superficial layer of a limited space of the tissue next to the peritoneal cavity. In this fact is to be found an explanation for the often noted "exceedingly imbibed and œdematous condition of the tissue, especially of the posterior walls" in the dead rabbits.

ON ABSORPTION OF STRYCHNINE AND HYDROCYANIC ACID FROM THE MUCOUS MEMBRANE OF THE STOMACH.—AN EXPERIMENTAL STUDY ON RABBITS.*

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It has always been, and still seems to be, the prevailing opinion that the stomach is capable of absorbing soluble and diffusible substances, and there seemed, indeed, to be no reason to suppose that the mucous membrane of the stomach should with regard to absorption be inferior to any other mucous membrane.

Recent investigations, however, have brought to light some new facts with regard to the power of absorption of the stomach, the most striking of which is surely the unexpected result that the stomach does not absorb any water. Edkins † introduced into the stomach of cats, after the ligation of cardia and pylorus, a measured quantity of salt solution, and recovered after an hour exactly the same quantity again. Von Mering,‡ who experimented on dogs with a duodenal fistula, found that a larger quantity of water came out through the fistula than the dogs had taken by the mouth. As my experiments have reference mainly to the absorption of strychnine and of hydrocyanic acid, I shall restrict my references to literature to those having direct bearing upon this subject. As far as I can ascertain,

* Read at the Eleventh Annual Meeting of the Association of American Physicians, April 30, 1896.

† J. S. Edkins, The Absorption of Water in the Alimentary Canal. *Journal of Physiology*, 1892, p. 460.

‡ Von Mering, Ueber die Functionen des Magens. *Therapeutische Monatshefte*, Mai, 1893.

there are only two statements on the absorption of strychnine in the stomach.

Bouley and Colin * have introduced strychnine into the stomach of animals whose pylorus was either ligated or paralyzed by vagotomy. They report that in the dog, swine, and cat the effects of the strychnine appeared very rapidly; the effect was retarded in the cow, and there was no effect—at least, no fatal effect—in the horse. Tappeiner † has experimented with strychnine exclusively upon cats, in whose stomach the poison was introduced through a stomach tube. A normal cat of about 2 kilos succumbed to the effects of 0.03 gramme of strychnine in an aqueous solution in eight minutes; cats whose pylorus was tied succumbed to doses of 0.05 and more only after an hour and thirty minutes or even later. If the strychnine solution, however, contained some alcohol, the strychnine took effect almost as rapidly as when the pylorus was not tied. Tappeiner also found that chloral hydrate in aqueous solution was not absorbed in the stomach of a dog with closed pylorus, while an alcoholic solution of it was readily absorbed.

Concerning the absorption of prussic acid from the stomach, I could find no reference.

My experiments relate to the absorption of various solutions in dogs and rabbits, and also in frogs, but I shall report here only the results I obtained in rabbits with strychnine nitrate and hydrocyanic acid. The animals were all well narcotized with ether during the operation. In most cases, after the introduction of the poison into the stomach, the pyloric and cardiac ends of the stomach were tied. At the pylorus the ligature was applied in the groove between the pylorus and duodenum, avoiding the inclusion of the vessels of the stomach, and at the cardia the ligature was applied on the œsophagus just beneath the diaphragm. I shall now let the experiments speak for themselves.

Experiment 3.—Rabbit, 1,800 grammes, not starved. Through a soft catheter, introduced by the mouth into the stomach, 10 milli-

* Colin, Traité de physiologie comparée, vol. ii, p. 91.

† Tappeiner, Ueber Resorption im Magen. *Zeitschrift für Biologie*, 1880.

grammes of strychnine were injected, catheter removed, and rabbit taken off from the board. After fourteen minutes a typical strong tetanus broke out; a few more followed, and the rabbit died.

Exp. 41.—Rabbit, 1,760 grammes, not starved. Through an opening made in the œsophagus a catheter was introduced into the stomach, and 6 milligrammes of strychnine in water coloured with methylene blue injected. Rabbit put on a box. After eighteen minutes, animal showed great uneasiness, and nine minutes later, with a sudden jerk, a strong opisthotonos set in, which lasted over two minutes. Rabbit remained hyperæsthetic for half an hour, and gradually recovered.

These two control experiments are sufficient to show that 6 to 10 milligrammes of strychnine brought into the full stomach of a rabbit do not fail to produce within half an hour the characteristic effect.

Exp. 4.—Rabbit, 980 grammes, starved three days, stomach nearly empty. Cardia tied, an opening made in duodenum, and a thin catheter introduced into the stomach, reaching the wall of the fundus. Sixty milligrammes of strychnine in 10 cubic centimetres of a forty-per-cent alcoholic solution, coloured with indigo carmine, were injected, the tube removed, duodenum and abdomen sewed up, and the animal brought to the box. The rabbit lived fifteen hours, and at no time was there any sign of hyperæsthesia, of increased reflex irritability, not to speak of a convulsion. The death was apparently due to peritonitis and aspiration pneumonia (" Schluckpneumonie "), but certainly not to strychnine poisoning. The stomach was considerably distended, and apparently contained more fluid than before the ligation.

It might be claimed that the explanation of the striking fact that 60 milligrammes of strychnine could remain for fifteen hours in the stomach without absorption is that by the ligation of the cardia and pylorus all the blood vessels and the lymphatics of the stomach were ligated, thus leaving no path open by which the poison could have been carried into the circulation. However, the stomach had by no means an ischæmic appearance; on the contrary, distinct pulsation could be seen also after the ligation, and the stomach retained in every respect its natural appearance. At any rate, such a claim can not be raised against the following experiment.

Exp. 7.—Middle-sized rabbit, pylorus ligated, cardia open; introduced through a catheter by the mouth 60 milligrammes of strychnine

in an aqueous solution. Rabbit lived nineteen hours without showing any effects of strychnine. Death and autopsy as in the preceding experiment.

Here the vessels of the cardiac end of the stomach remained open; nevertheless no absorption took place.

Exp. 28.—Rabbit, 1,200 grammes, starved two days, cardia left open; introduced a tube through the duodenum into the stomach; injected into the fundus 45 milligrammes of strychnine; rabbit remained for an hour on the holder; no sign of any effect. Then the rectum was tied, and by a hypodermic syringe 3½ milligrammes of strychnine, coloured with indigo carmine, were injected through the wall of the rectum into the impacted lumen. After twelve minutes a tetanic outbreak set in, which terminated fatally. The tetanic outbreak was apparently due to the quick absorption of the 3½ milligrammes of strychnine from the rectum. No drop escaped into the peritoneal cavity or infiltrated the wall of the rectum, as could be seen by the absence of the blue colour.

Against the conclusion that the mucous membrane of the stomach of the rabbit does not absorb strychnine, another objection could be raised, namely, that the stomach of the rabbit is never thoroughly empty, and that the strychnine in all the cases is enveloped by the remnants of the food and does not come in contact with the mucous membrane at all. To meet this objection, the following experiment was made:

Exp. 35.—Rabbit, 1,200 grammes, starved sixty hours, stomach quite empty. By a catheter, which was introduced through an opening in the œsophagus, 100 cubic centimetres of water were brought into the stomach, catheter removed, and cardia left open. A tube was introduced through duodenum and pylorus; the inner end of this tube was brought in close proximity with the wall of the stomach, then 60 milligrammes of strychnine, deeply coloured with indigo carmine, was injected, and some air blown in; now the tube was removed and the pylorus tied. An hour and twenty minutes passed by without a sign of a strychnine effect. Judging from the manifold experiences I had had I was sure that if the rabbit did not show the effects of the strychnine in the first hour the effect would not appear at all. Therefore the rabbit was used for another experiment. Three milligrammes of strychnine were

injected through the anus into the rectum. After eight minutes a brisk tetanus carried the rabbit off. At the autopsy the entire stomach was taken out. The stomach was transparent, the level of the fluid was distinctly visible and changed easily with each change in the position of the stomach. On opening the stomach, the contents flowed out easily, no food clung to the mucous membrane, and where the coloured strychnine touched the mucous membrane there were distinct marks of the indigo carmine superficially adherent to the epithelial layer.

In this experiment the strychnine was surely in close contact with the mucous membrane of the stomach, and the vessels of the cardiac end were also not ligated; nevertheless no absorption took place.

Exp. 40.—Rabbit, 2,100 grammes, cardia tied; tube passed through duodenum into the pyloric part of the stomach; injected 50 milligrammes of strychnine, coloured with indigo carmine, directed to the front wall of the pyloric part; this part bulged up, tube removed, and pylorus tied. After two hours and five minutes, still no effect. Now 5 milligrammes of strychnine, coloured with methylene blue, was injected into the front wall of the stomach between the muscularis and the mucosa; a blue-stained area appeared, but no drop of the fluid escaped. After two minutes, a stormy tetanus broke in like lightning, and the rabbit was dead.

This shows strikingly that the ligation of the cardiac and the pyloric ends of the stomach does not prevent in the slightest the lymphatics and the blood vessels from carrying into the circulation the fluid which reaches them.

In sharp contrast to our results with strychnine, we found that the stomach of the rabbit promptly absorbs prussic acid.

Exp. 6.—Large rabbit, not starved, cardia tied; tube introduced through the duodenum into the stomach; injected 100 milligrammes of hydrocyanic acid (5 cubic centimetres of the official two-per-cent solution), coloured with indigo carmine; tube removed and pylorus tied. Rabbit soon shows uneasiness, the breathing becomes laboured, clonic convulsions set in, and the animal dies in fifteen minutes.

Exp. 26.—Rabbit, 2,200 grammes, not starved, cardia ligated; tube passed through duodenum; injected into the fundus 60 milligrammes of

prussic acid; tube removed and pylorus tied. Rabbit dies after twelve minutes in the same manner as in the former experiment.

At the autopsies of some of the rabbits which died from prussic acid, distinct hæmorrhagic surfaces were found on the mucous membrane of the stomach. This probably accounts for the good absorption of the prussic acid. The fact that hydrocyanic acid was constantly absorbed from the ligated stomach of the unstarved rabbits shows that the acid easily reaches the mucous membrane even in a full stomach, and, further, that the ligation alone does not prevent the blood vessels and the lymphatics from absorbing fluids which come within their reach. I have, therefore, in a few experiments with strychnine used the absorption of hydrocyanic acid as a test for the points in question.

Exp. 12.—Very large rabbit, 3,200 grammes, starved twenty-four hours, cardia tied; introduced tube through duodenum into the stomach, and pylorus tied tightly around tube. At 5.12 P. M. injected 50 milligrammes of strychnine in 10 cubic centimetres of water; at 8.28 rabbit perfectly quiet; injected again 50 milligrammes in 10 cubic centimetres of alcohol (fifty per cent). At 9.28 rabbit sleeps well; no sign of strychnine effect. Injected now 100 milligrammes of prussic acid in an alcoholic solution; rabbit soon starts breathing irregularly; short inspirations; convulsive expirations, with long intermission; heart first slow, then very rapid, growing imperceptible; some clonic convulsions; no hyperæsthesia; no tetanic or tonic contractions; rabbit dies gradually within twenty minutes.

Exp. 42.—Rabbit, 1,740 grammes, cardia tied; tube through duodenum into stomach; injected 200 milligrammes of strychnine in 20 cubic centimetres of water; tube removed and pylorus tied. After an hour and fifty minutes no effects whatsoever; 60 milligrammes of prussic acid, coloured with methylene blue, were now injected by means of a hypodermic needle, thrust through the front wall of the stomach, directly into the mass of food; the fine opening clamped; nothing escaped. Very soon after the veins on the walls of the stomach present a bright brick-red colour; the same was seen on the edges of the spleen; irregular laboured breathing set in; some clonic convulsions appeared without the presence of any hyperæsthesia; the rabbit died within twelve minutes.

The death was certainly due to the prussic acid, which rapidly reached the mucous membrane, penetrated it, and was carried off by the lymphatics and the blood vessels into the circulation. The enormously large dose of strychnine has probably also reached the mucous membrane, and would also have been carried into the circulation if it could only penetrate the epithelial layer to reach the lymphatics and the blood vessels.

I append two more experiments to show the character of absorption in some other parts of the alimentary canal.

Exp. 23.—Large rabbit, cardia tied; tube passed through duodenum into the stomach; injected 40 milligrammes of strychnine in a thirty-three-per-cent alcoholic solution; tube removed and pylorus tied. After eight hours no effect. Then 14 milligrammes of strychnine were injected into the œsophagus, the upper end of which was tied. After an hour and thirty-five minutes, still no effect. Now 4 milligrammes of strychnine were injected into the pharynx; in four minutes a brisk tetanus broke in, which ended fatally. Of course, it can not be stated whether the absorption has taken place only within the pharynx or also in the mouth, and perhaps even only in the mouth alone, though the injection was made directly into the pharynx.

Exp. 19.—Middle-sized rabbit, œsophagus tied at the upper end and also below the diaphragm. The vagi and the inferior laryngeal nerves were avoided. Through a cannula tied in the œsophagus several injections were made. At first only 4 milligrammes of strychnine were injected; half an hour later 4 milligrammes more were added, and forty minutes after this again 10 milligrammes were injected. An hour and fifteen minutes passed after the last injection with no sign even of increased irritability. Now 4 milligrammes of strychnine were injected into a loop of the small intestine; after seven minutes there was already marked hyperæsthesia, and five minutes later a stormy tetanus caused death.

I have to add that in one experiment half an hour after injection of 10 milligrammes of strychnine into the œsophagus, a tetanic attack did take place, but the œsophagus in this case was very much distended by air, which surely facilitates the absorption.

The results of my experiments, briefly summed up, are as follows:

The introduction of 6 to 10 milligrammes of strychnine into the full stomach of a rabbit with pylorus open is sure to bring on a tetanus within a short time. The effect is probably brought about by the rapid passage of the strychnine from the stomach into the intestine, whence it becomes absorbed in a few minutes.

When the pylorus, however, is closed, even such large doses as 200 milligrammes of strychnine, remaining for many hours within an empty stomach, with good circulation and with intact innervation of the vagi, do not produce any effect at all. It seems that the mucous membrane of the stomach does not absorb even the smallest fraction of the strychnine. Next to the stomach, we also found that the mucous membrane of the œsophagus absorbs strychnine very poorly.

The pharynx is apparently the part of the alimentary canal which absorbs best; 3 to 4 milligrammes in the pharynx will induce a tetanus in three to four minutes.

The rectum absorbs nearly as well as the pharynx, as 3 milligrammes injected through the anus will call forth a tetanus in six to eight minutes. The absorption seems to take place here even better than in the small intestine, as it takes twelve to fifteen minutes before a tetanus breaks out after an injection of 4 to 5 milligrammes of strychnine into a loop of the small intestine.

Prussic acid is absorbed very well from the stomach even when the pylorus is ligated. It seems to produce a hæmorrhagic surface on the mucous membrane, which facilitates the absorption. Possibly, also, the volatility of prussic acid may favour prompt absorption, as gases seem to be readily absorbed in the stomach.

THE PRODUCTION OF ANTITOXIN BY THE PASSAGE OF ELECTRICITY THROUGH DIPHTHERIA CULTURES.

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AND
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Prochownik and Späth, in 1890, made the observation that bacteria adhering to the positive pole of an electrical current are retarded in growth or killed, according to the strength and duration of the current. In these experiments results were obtained only by growing the bacteria in agar on the electrodes themselves, and then dipping these in salt solution and passing the current. Simply passing the current through a liquid culture or suspension of the bacteria had no effect. They attribute the effect to the chlorine gas liberated at the positive pole.

Apostoli and Laquerrière found that passing a current through bouillon cultures had the effect of killing bacteria if the poles are placed close together. The action is independent of the heat that is produced, and takes place only at the positive pole. The action is believed to be due to the decomposition of the nutrient solution into acids and to the liberation of oxygen. The electricity itself is not believed to play any part directly.

Foth found that electrical currents lessen the fermentative power of yeast cells. He concludes that the action is due to the products of electrical decomposition, notably to the development of ozone.

Tolomei found that only strong discharges from a Ruhmkorff apparatus, placed very close over the surface of the liquid, stops acetic-acid fermentation. These discharges do not cause a sterilization of the liquid, and fermentation begins again slowly after the discharge.

Spilker and Gottstein tested the effect of electricity upon cultures

and suspensions of bacteria in various fluids. The bacteria were destroyed in suspensions in water and in "weiss Bier," but not in milk. The suspensions in blood were sterilized by the electrical current, and it was supposed that the iron in the blood had something to do with the action; but suspensions in solutions of various salts of iron were not sterilized by the electrical current. Suspensions in solutions of iron albuminate were retarded in growth, but development of bacteria recommenced after eight days.

Verhoogen found that there was a destruction of bacteria around the positive pole with weak currents, but if the current is made strong enough there is this action also around the negative pole.

Charrin reported some observations of Gautier made on suspensions of bacillus pyocyaneus in solutions of iodide of potassium and in artificial serum. The positive electrode consisted of copper, and was dipped into the suspensions of the bacteria. In both cases the bacillus was deprived of its power of producing any colour when grown on agar after the current was passed for seven minutes; longer action was found to retard subsequent growth and even to cause sterilization.

Krüger found that the constant current is able to suspend growth, but not to kill bacteria, where all chemical action due to the products of decomposition of the nutrient material is excluded. Cultures subjected to a certain strength of current for a certain length of time possess immunizing properties.

Smirnow inoculated a large number of rabbits with 0.5 to 0.7 cubic centimetre of a bouillon culture of the diphtheria bacillus two or three days old, and twenty-four hours afterward, when the animals were very sick, he injected 8 to 10 cubic centimetres of the product from the positive pole of electrolyzed toxin. The animals all recovered. In order to convert 200 cubic centimetres of toxin into antitoxin, he finds it necessary to use a current of 80 milliampères for not less than sixteen to eighteen hours. The action of the current on the toxin is apparent at the positive pole by a bleaching of the bouillon; at the negative pole by a darkening of the bouillon. In applying the current, Smirnow used a U-shaped tube, with a glass stopcock in the middle.

Krüger finds that the calibre of the U-tube used to contain the culture should not be more than 18 millimetres, and that the entire length should not be more than 22 centimetres. The current should be made to pass upward through the liquid. Krüger, moreover, obtained the best results by scraping the diphtheria bacilli off of agar cultures and making a suspension in one-per-cent salt solution. This he finds better than bouillon cultures. He was able to prevent death in rabbits inoculated with virulent cultures by injection of 3 cubic centimetres of cathode product, and he found he could save rabbits with 2 cubic centimetres of the crude product.

Smirnow finds that diphtheria antitoxin, which cures inoculated rabbits and guinea-pigs even in advanced stages of the disease, can be obtained by electrolysis; that the strength of the antitoxin depends largely upon the strength of the toxin that has been subjected to the current; moreover, that it depends upon the degree of acidity of the liquid at the negative pole. This acidity should be such that 0.9 to 1.3 cubic centimetre of normal soda solution neutralizes 1 cubic centimetre of the electrolyzed product. This degree of acidity is the optimal for guinea-pigs, but for rabbits the current must be allowed to act for a longer time and the acidity should be such that 1.5 to 1.7 normal soda solution is required to neutralize it. He finds that any of the electrolyzed product above that which is just sufficient is injurious.

D'Arsonval and Charrin find that continued or intermittent currents of high potentiality attenuate toxins as well at the positive as at the negative pole. They find that toxin treated with a continuous current is simply destroyed by the chemical products of electrolysis, and possesses little or no antitoxic properties. But toxin treated with an intermittent current, where the current was reversed about 200,000 times a second, produced fairly strong antitoxin in a quarter of an hour. They conclude that the production of antitoxin by an electrical current is due to a violent vibration of the molecules, resulting in a rearrangement, and not to any chemical decomposition.

In the following tests we employed a current from the Edison electric light, which usually has a strength of 110 volts; this was re-

duced and controlled by a simple rheostat inserted in the current. The apparatus used to hold the liquid cultures consisted of two test tubes, connected about the middle by a tube with a stopcock. In some cases the stopcock was of glass, in others of hard rubber. The resulting shape of the apparatus is that of the capital letter H, the vertical arms open at the top and closed below with a platinum-wire electrode melted in. In order to expose a large surface, the wire projects inside the tubes several inches, and is coiled up. The cross arm of the H carries the stopcock. Following Krüger's suggestion, the length of the lower half of the vertical arms and the transverse connection together were made to measure 22 centimetres, and the diameter 19 millimetres. The apparatus with these dimensions gave better results than a larger apparatus made after a similar pattern with a hard-rubber stopcock. In all cases the current was made to pass upward through the liquid. The strength of the current was determined with a Weston milliampèremeter.

The action of the current is made evident by the evolution of gas bubbles, which are given off from the electrodes, and rise and form a foam at the top. A precipitate quickly forms in both tubes, but more abundantly over the positive pole, and the liquid in this tube also becomes lighter in colour. After the stopcock is turned off, cutting off the current, the precipitate soon settles to the bottom. The fluid over the negative pole becomes darker in colour. There is a peculiar odour given off, resembling that of chloride of lime; but it was found that the fluid contained only one tenth of one per cent of chlorine. The strength of the current increases gradually as it is allowed to pass, and has to be regulated with the rheostat from time to time.

Tests were made on bouillon cultures of the diphtheria bacillus grown for varying lengths of time in the incubators. Some of them were filtered through several thicknesses of filter paper, and some of them were unfiltered. The current usually employed varied from 44 to 72 milliampères, and was allowed to act, as a rule, for two hours, though some tests were made with cultures that had been subjected to the current for longer and shorter periods than this. In all cases the strength of the cultures was tested on guinea-pigs previous

to the passage of the current. The tests as to whether antitoxic property was developed by action of the electric current were made by Behring's method, i. e., by administering ten times a certainly fatal dose of diphtheria toxin along with varying amounts of the electrolyzed products, either mixed with the toxin or injected at a different place in the animal.

These tests soon showed that there is a production of antitoxin over the positive pole, as the following result of one of several experiments will show:

A guinea-pig, weighing 230 grammes, was inoculated under the skin with a mixture of 1 cubic centimetre of diphtheria toxin and 5 cubic centimetres of the product from the positive pole. A similar guinea-pig was inoculated at the same time with 1 cubic centimetre of the same toxin mixed with 5 cubic centimetres of the product of the negative pole. Before the current was passed the toxin was found to be of such strength that 0.3 cubic centimetre would kill an average-sized guinea-pig in forty-eight hours. The toxin used to mix with it after the passage of the current killed in doses of 0.1 cubic centimetre in forty-eight hours. The guinea-pig inoculated with the mixture containing the product from the positive pole remained alive, and showed no symptoms of disease. The other animal died promptly in forty-eight hours. Two and a half cubic centimetres of the electrolyzed toxin was insufficient to neutralize ten times the minimum fatal dose of toxin. In order to test whether the product of the negative pole was in itself poisonous, a guinea-pig was injected with 5 cubic centimetres of it, and remained perfectly well; so the toxin properties are destroyed at the negative pole, but no antitoxin is developed.

The strongest antitoxin we were able to obtain was such that 2 cubic centimetres neutralized ten times the minimum fatal dose for guinea-pigs. In a single experiment made on rabbits by intravenous injection, neither 2 cubic centimetres of the product of the negative pole nor 3 cubic centimetres of the positive pole counteracted 0.6 cubic centimetre and 0.5 cubic centimetre respectively of toxin.

It occurred to us to try whether the presence of the bacilli them-

selves would influence the result. So unfiltered cultures and the sediment in old bouillon cultures were tried, but the result was the same, viz., 2 cubic centimetres of the product from the positive pole counteracted ten times the minimum fatal dose, while the same amount from the negative pole had no effect.

Tests with a much weaker current for a somewhat longer time seem to indicate that this method offers an advantage. But if the current, even though very weak, be kept up very long—for example, for a week or more—the antitoxin seems to be less potent.

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DIPHTHERIA ANTITOXIN SOMETIMES FOUND IN THE BLOOD OF HORSES THAT HAVE NOT BEEN INJECTED WITH TOXIN.

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Every one who has undertaken the production of diphtheria antitoxin has encountered a marked difference in the behaviour of the horses undergoing inoculation. With some horses it is a matter of very little difficulty to obtain antitoxin of high potency in a comparatively short time. With others it seems impossible in any way to obtain strong antitoxin, and with others again there is such a violent reaction even with small doses of toxin that it is impossible to continue inoculation.* It occurred to me that the explanation of this might be found in the normal differences of the blood in the different animals. In order to test this, all the horses that were afterward used for the production of antitoxin for the laboratory of the Board of Health of Philadelphia were first tested as to the presence of antitoxin normally. It was, perhaps, to be expected that marked differences in this respect would be observable. I had rather expected, if there were any differences, that these would be gradual, that all horses might exhibit more or less antitoxic power in their blood. But from the results of the tests I am inclined to believe that this is not the case. There is in some cases a notable amount of antitoxin, in others it seems to be totally lacking. It seems to be present to a certain definite extent, or to be entirely absent.

In all, the blood-serum of twelve healthy horses was tested previous to injection with diphtheria toxin. Out of this number three

* This difficulty has now been entirely overcome by injecting these supersensitive horses with antitoxin previous to and during the treatment with toxin, as recommended by Pawlowsky and Maksutow, *Zeitsch. f. Hyg.*, vol. xxi, p. 485, 1896.

were found to possess antitoxin of such strength that 3 cubic centimetres of the serum were capable of neutralizing ten times the minimum fatal dose for guinea-pigs, i. e., $^1/_{30}$ of a normal antitoxin unit. In all three horses the strength of the antitoxin seemed to be the same. The guinea-pigs injected with 3 cubic centimetres all remained alive and well; those injected with a lesser amount all succumbed promptly to the accompanying dose of toxin. Injections of blood-serum from horses not possessing normal antitoxin exerted no influence even in larger doses up to 5 cubic centimetres. The difference seems to be clearly one of quality and not of quantity, an essential difference in some constituent of the blood.

The behaviour of the horses possessing antitoxin normally in their blood presents certain points of interest on injections of toxin. These horses show much less reaction at the beginning than other horses, and it is possible to increase the doses to a certain point much more rapidly without causing alarming symptoms. After the dose has been increased till it reaches about 100 cubic centimetres or more the reaction seems to be as marked as in those horses that have had to be brought up to this dose more gradually. Experiments on two horses with antitoxin in their blood normally and two without antitoxin were begun together. One of the latter reacted so strongly that it was impossible to continue even with very small doses. The experiments on the other three horses have been continued. Up to the present time one of the horses, with antitoxin normally present, has received nearly 3 litres of toxin, the other over 4 litres, and the horse without the antitoxin normally present has received less than 2 litres, and yet they all show about the same amount of reaction at present from injections of 100 cubic centimetres of toxin. At first, when the doses were small, there was practically no reaction in the horses where the antitoxin was present normally, whereas in the other horse there was always decided reaction.

One of the horses with antitoxin normally present (the horse that has received about 3 litres of toxin) is beginning to furnish strong antitoxin, almost 1,000 units to 10 cubic centimetres. The other horse with antitoxin normally present (the horse that has received

over 4 litres) furnishes very weak antitoxin, less than 600 units to 10 cubic centimetres. The horse * that had no antitoxin normally, that had received nearly 2 litres of toxin, furnishes quite strong antitoxin, almost 1,000 units to 10 cubic centimetres. It would seem from this that the presence or absence of more or less antitoxin normally has no effect upon the ultimate production of artificial antitoxin by inoculation, but its presence enables the inoculations to be made with less risk to the animal.

It is difficult to explain the presence of antitoxin normally in the blood of horses. Wassermann † found antitoxin in the blood of healthy persons, who claimed never to have had diphtheria, but he inclined to the belief that these persons at some period must have suffered from unrecognized diphtheria. Orlowski ‡ also found diphtheria antitoxin in the blood of children who had presumably never suffered from diphtheria, and he found it also in the blood of convalescents, as others had done. Orlowski does not accept Wassermann's explanation, because he does not find that the age of the children has anything to do with the presence of normal antitoxin, and, moreover, he finds that the antitoxin present after convalescence disappears.#

* This animal has since died.

† Wassermann, Die Immunität Gesunder gegenüber Diphtherie. *Deutsche med. Wochenschr.*, 1894.

‡ Orlowski, Ueber die antitoxischen Eigenschaften des Blutserums bei Kindern. *Deutsche med. Wochenschr.*, 1895.

Cobbett (Contribution to the Study of the Serum Therapeutics of Diphtheria, etc. *The Jour. of Path. and Bact.*, vol. iii, No. 4), in an article which appeared since the above was sent in for publication, has also found that there is a definite amount of antitoxin present normally in the blood of some horses. He furthermore finds that, if there is no antitoxin in the blood, injection of the latter into guinea-pigs rather increases than diminishes the action of diphtheria toxin.

THE INFLUENCE OF EXERCISE ON GROWTH.

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The effects produced by exercise on the human body may be divided into immediate and remote, subjective and objective, ponderable and imponderable, permanent and temporary, real and imaginary.

The principal immediate, subjective, and temporary effect of almost all forms of exercise is a general feeling of well-being. It is quite immaterial whether the exercise which is being indulged in consists in walking, running, bicycle or horseback riding, playing football or lawn tennis, rowing in a shell, or doing gymnasium work of any kind, while in the midst of it, and while the supply of those substances in which our energy is stored up in the muscles is still equal to the demand or before breathlessness puts a stop to the enjoyment of our work, we invariably experience a certain sense of exhilaration, which must be actually felt in order to be properly appreciated. This fact also explains in a way the quite peculiar and often even startling enthusiasm of a great many people for the particular form of exercise during the taking of which they first experienced this feeling.

As an immediate direct consequence also of almost all forms of exercise, it is perfectly true that we sleep better and think clearer; we react, discriminate, and associate ideas quicker; we see, hear, and taste more distinctly; the functions of the skin and kidneys are increased; digestion and assimilation are greatly improved; the expansion of our lungs is greater and the contractions of the heart are stronger, blood pressure being slightly increased; and, finally, all the muscles, both voluntary and involuntary, contract more quickly and with greater effectiveness. Such effects as these have been abundantly recorded.

Now, whatever may be the more scientific explanations in detail which physical and chemical physiology and psychology have to offer for this temporary simultaneous increase in the functions of all these different organs and tissues, resulting from vigorous exercise of any kind, it remains an interesting fact. An increased supply of oxygen in the capillaries, together with a more rapid and abundant outflow of the products of wear and waste than takes place under the conditions of rest, seem to constitute the common primary cause of it all. The condition may perhaps not inaptly be compared with the simple shifting of the lighted candle from the normal atmosphere into one of oxygen. As the candle burns with greater brilliancy, as its wick is consumed more completely, and combustion is more thorough when in an atmosphere of oxygen than it would be if merely surrounded by ordinary air, so also during exercise must all those chemical processes be improved and quickened upon which depend not only the quality but also the quantity of the functions of the different cells of the body whenever brought in contact, as is the case during exercise, with an amount of oxygen from two to seven times greater, for a given time, than under conditions of rest, and when, furthermore, the products of such increased combustion are carried off so much more rapidly than is the case under normal conditions.

Such general effects as these, directly traceable to almost any form of vigorous exercise, are simple matters of daily experience, and every one of us has, no doubt, experienced and verified them frequently.

It is, however, quite different with regard to some of the more remote, objective, and permanent effects of exercise. The methods that must be employed in order to ascertain and define these precisely and with sufficient scientific accuracy, so as to make them absolutely convincing, must be more carefully chosen than has been done heretofore, and must rest upon a more substantial basis than do those that usually suffice for the determination of the effects of a merely subjective and temporary character.

Thus, for instance, it is not deemed sufficient to note or record

the various increases or changes in several different dimensions that take place during or after a course of exercises taken in a gymnasium in the cases of a number of persons, and which changes are usually determined by two sets of measurements taken before the course begins and after it is ended, for in the various increases or changes noted after this manner are contained not only those values that *may* be due to the exercise performed, but also those that *must* be due to the natural growth and development, and which are quite naturally expected to occur during the period of life occupied by the persons who are the subjects of such observations, as well as by the time occupied by the observations themselves.

In order, therefore, to determine exactly what increment of height, weight, lung capacity, or strength is due to exercise, we must first of all know what the amount of normal growth in these dimensions is that is expected to take place during such time as was occupied by the exercise and for the same period of life.

In other words, the absolute annual growth values for each and all the different dimensions must first of all be worked out before we can possibly deduce those values which may result from a certain given amount of exercise and before, it would seem, we had any very valuable reason or right at all to talk of the effects on growth and development of any particular form or system of exercises.

Now, normal-growth tables and curves in several dimensions of children of both sexes, and from the sixth to the eighteenth year of age, have been worked out by Bowditch* and Porter.† From the ages of sixteen to twenty-two years such tables and curves have furthermore been calculated and published from the records of 4,537 naval cadets.‡ It would, therefore, seem that we are in possession of a certain amount of valuable statistical material covering a period of normal growth from the sixth to the twenty-second

* The Growth of Children. *Twenty-second Annual Report of the State Board of Health of Massachusetts.*

† The Growth of St. Louis Children. *Trans. Academy of Sciences, St. Louis*, vol. vi, No. 12.

‡ The Growth of United States Naval Cadets. *Proceedings U. S. Naval Inst.*, vol. xxi, No. 2.

year of age, and which material is well calculated to serve the above-mentioned purposes.

The values of the absolute annual growth that are shown in these tables represent, of course, the averages for the number of subjects that were available for measurement between the different years. The figures given under the different percentile grades are likewise only average values. These average values naturally tell us nothing about the peculiarities in growth that are, without doubt, inherent in distinct types, but which it was impossible to eliminate and to study separately. Since, however, it may well be taken for granted that the racial composition of a nation changes but slowly, these average values may be expected to stand in a constant ratio to other averages derived from the same nation or parts of it, and consequently they may be safely employed for purposes of comparison with values derived by the same methods and obtained from identical sources. At any rate, these values, although perhaps not absolutely true, are the best that it is possible to obtain with the means at present at our command. Pure types of man in these modern times are the exception rather than the rule, and no country with a modern civilization exists which is free from composite types. So, then, while we freely and frankly admit that our averages are far from representing the American types, and while the growth curves constructed from them can not be true for every individual included in these averages, they may safely be pronounced true for the great majority of them, and consequently they are and must be comparable as such, if not to individuals, at least to averages derived by similar methods and from identical sources.

One of our objects is to utilize these averages for the purpose of showing whether or no our method of physical training which was introduced at the Naval Academy about three years ago has an influence on the growth of naval cadets as regards height, weight, lung capacity, and general strength over and above that method which was in vogue before that time.

To this end we began about three years ago collecting data somewhat as follows: Every cadet entering the Academy was accu-

rately measured, all the measurements being taken the latter part of September of each year. The gymnastic work during October and November consists mainly in a daily setting-up drill, and from December 1st to the last of March a regular systematic course in gymnasium work, leading from the lightest calisthenic exercises very gradually up to heavy gymnastics and apparatus work, is gone through with. The work is done in the form of drills, five times a week, beginning at 4.15 P. M. and lasting three quarters of an hour each. All the exercises have been carefully selected and arranged according to physiological principles, and the drills take place under my personal supervision. Special exercises are also taken by those of the cadets in whom previous examination revealed the existence of special defects as regards symmetry, so that it may be said that during the first six months, the only time during which gymnastic exercises are made compulsory at the Academy, such exercises are administered on the proper principles and under due and proper supervision.

The last of March all those of the cadets who have not dropped out of my original list by resignation or dismissal from the service are re-examined, and the results of such examinations are recorded, as was done in the first examination. The difference between the first and second examinations, it may be fairly assumed, is made up of the values of the absolute semi-annual growth in height, etc., plus that amount which is due to gymnastic exercise, other conditions of life being equal for all the cadets under observation and those from which the normal averages had been worked out, and with which alone we propose to compare our gymnasium averages.

The ages of the cadets entering the Academy vary from sixteen to twenty years, the average during the last four years having steadily remained at eighteen. We have then a period of growth to deal with which covers four years—namely, from sixteen to twenty years. Dividing now my one hundred and eighty-eight observations that have so far accumulated, and arranging them according to age, multiplying, furthermore, by two these semi-annual values obtained, thus converting them into annual ones, they are ready for being compared with the tables giving the normal annual

absolute growth in the same dimensions, and which were calculated from 4,537 cadets of previous years.

So far as height alone is concerned, I am in the fortunate position of being able to make a still more valuable comparison. Being in possession of the complete annual records of the heights of one hundred and eighty-six cadets for the ages of from sixteen to twenty-two years, I was able to produce a table according to the individualizing method, and which, therefore, is of far greater value than the one obtained by means of the generalizing method, in spite of the numbers in the latter being much larger.

The possession of these two tables, both showing the normal amount of growth in height, but derived according to different methods, enables me not only to compare them both with those obtained from the gymnasium records, but also to test, in a measure, the comparative value of the two above-mentioned methods by which the two tables showing the normal values were produced. In comparing these two tables with each other, the first thing that will be noticed is that the distribution of the annual growth values is very different in the two; but when we add these values together in each table separately, their respective sums are absolutely identical, being in both sixty-nine respectively. I am therefore inclined to believe that the sums in each and both are correct as applying to the material in hand. Moreover, the fact that the distribution of the values in the table produced by the individualizing method is so much nearer the truth and to nature than that shown in the other table proves conclusively that the individualizing method is the only one that will ever give us the correct values of the absolute annual growth that occurs between any two successive years of life. On examining the figures representing the averages derived from the individual records of one hundred and eighty-six cadets, and comparing them with those obtained from one hundred and eighty-eight cadets that were under observation in the gymnasium, a very striking similarity with regard to the distribution of the annual growth values may be noticed at once.

These two tables, graphically represented, would show two nearly

parallel lines, a circumstance arguing strongly in favour of the conclusions drawn from a comparison made between them.

It would appear from these figures that a positive increase in height may be attained through properly systematized gymnastic exercise, when administered between the years sixteen and twenty, amounting to 26·6 millimetres over and above that which may be expected to take place without such exercise. (See Table I.) The largest amount of gain in height occurs between sixteen and seventeen years, being 38 millimetres, or 10 millimetres more than the normal increase. This is in perfect accord with the laws of growth, for it is well known that the younger a person the greater also are his chances for growth, and it would seem a most natural inference to make that any agent influencing growth favourably would likewise have its best chance to assert itself at that time. For the remaining years, or from seventeen to twenty-one, a surplus of about 4 millimetres is steadily gained over the normal values.

It is perhaps not altogether superfluous to mention here that all the cadets before coming to me for their first measurements have had at least one month's setting-up drill, swimming, and infantry drill. About one half of their number entering during the month of June have, of course, had these same drills for a much longer time. All of them have, furthermore, passed a satisfactory physical examination before a board of experienced medical examiners. The material on which these examinations or observations were made may be safely accepted as normally developed and well set up at the time of their first examination by me.

TABLE I.—*Height.*

		AVERAGE INCREASE IN MM. BETWEEN THE YEARS					
		16–17.	17–18.	18–19.	19–20.	20–21.	Total.
1	From records of 4,537 * naval cadets, normal values.....	28	6	15	19	1·0	69·0
2	From records of 186 continuous individual cadets, normal values.	28	20	12	7	2·0	69·0
3	From records of 188 cadets influenced by gymnasium exercise.	38	24	16	11	6·6	95·6

* In this number are included several hundred who left but the records of one examination; they were afterward rejected in the mental examination, and, consequently, never became cadets.

The total amount of increase in height to be deduced from the adjoining table being 26·6 millimetres, or a little more than one inch, is, perhaps, not quite up to the true gain in this dimension, but rather a little below it, our normal standard having been obtained from naval cadets whose training at the Academy as well as on board ship implies an amount of exercise over and above that which is implied in the education of the average individual at either the high school or college, and from the effects of which it was, of course, impossible to disentangle our normal averages.

Now, a gain in height of one inch during four years of systematic exercise may perhaps not seem a very great reward for the time spent. On further thought, however, it is of the greatest importance when applied to an entire nation or race, whose very existence may depend on the physical resources and strength of its individual members, as it sometimes does even among the most civilized. The struggle for existence, in the physical sense of that term, has not yet come to an end, and modern civilization, instead of preparing for this end, seems, on the contrary, to be preparing man for still greater and graver struggles in the near future than he has had to encounter in the past.

Throughout these observations it was clearly shown * that the taller a cadet was at the beginning, the greater also was the amount of weight and strength that was gained by the exercise he was made to perform. It was invariably observed in every series of observations that those of the cadets whose gain in total strength was over one hundred kilos were also taller and heavier than those whose gain in this respect fell below one hundred. An increase in height, therefore, means a corresponding increase in weight as well as strength. Consequently we incline to the opinion that height is the most important consideration in an investigation of this character, and any agent that influences growth in height in man influences growth in bone. Growth in height practically ends at the age of twenty-one years, while weight and strength may be increased al-

* See *Reports of the Surgeon General to the Secretary of the Navy* for the years 1893, 1894, and 1895.

most indefinitely and to a much later age, and when we speak of the growth of man in a general sense we always imply growth in height.

Both Bowditch and Porter have shown that the period of the most rapid growth for boys occurs between the ages of thirteen and fifteen. Our observations begin with boys of sixteen years of age, a time when the period of the most rapid growth for the average boy has passed. Now if, as is clearly shown by our table, it is possible to add one inch to our height by means of systematic exercise after the age of sixteen, the inference is rendered very probable that we could add still more to it if such exercise were applied earlier. This must be equally true for growth in the other dimensions.

TABLE II.—*Weight.*

		MEAN INCREASE IN KILOS BETWEEN THE YEARS					
		16–17.	17–18.	18–19.	19–20.	20–21.	Total.
1	Records of 4,537 naval cadets, normal values	3·6	3·2	2·4	0·7	0·2	10·1
2	Records of 188 observations in gymnasium.....	8·8	6·6	6·6	6·6	6·4	35·0

Examining the figures presented in the second table, we find that a very decided increase in weight has taken place, which increase is due to exercise. The amount of increase is greatest between the years sixteen and seventeen, just as was the case with regard to height, but, unlike what was observed in the table of the heights, the various additions of weight due to exercise remain practically uniform for each year from the seventeenth to the twenty-first.

The sum of 35 kilos, or about 77 pounds, is therefore considered a much larger proportionate amount of increase for weight than was noticed with regard to height. This shows well how much easier growth in weight is influenced by exercise than is height. Weight, on the other hand, is likewise easily and quickly lost. Height, once attained, is rarely, if ever, lost again.

The figures in Table III show clearly that an increase in lung capacity has occurred above the normal amount between each year

from the sixteenth to the twenty-first. The respective annual values are steadily diminishing from the sixteenth to the twenty-first year in both the normal records and those obtained in the gymnasium; they stand, therefore, in a more constant ratio to the various values presented in the table of the heights than to those given in the table of the weights, which remain practically uniform from the seventeenth year on to the twenty-first.

TABLE III.—*Lung Capacity.*

		MEAN INCREASE IN LITRES BETWEEN THE YEARS					
		16–17.	17–18.	18–19.	19–20.	20–21.	Total.
1	Records of 4,537 cadets, normal values	0·250	0·167	0·152	0·083	.....	0·652
2	Records of 188 observations in gymnasium	0·658	0·540	0·462	0·376	0·340	2·374

This must be regarded as an observation of some importance, since it has a bearing on the "vital index" of Demeny, or the ratio of lung capacity to weight $\left(\frac{LC}{W}\right)$. A high vital index is deemed an attribute of perfect training. In order to attain a high vital index, the lung capacity must show a greater proportionate increase than the weight, for the greater the capacity of the lungs and the less the weight of a person, the higher will be this index. Thus, for example, the mean vital index, calculated from the records of 4,537 cadets, was found to be 0·077 at the age of twenty-three years, which is considered a very high index.

From a physiological point of view, nothing would appear more rational or more desirable than to acquire a proportionate addition to our lung area for every pound of increase in muscular substance which we gain during the exercise which we take. Comparing now the figures presented in Tables II and III, respectively, it will be noticed, while the various additions to weight (not necessarily all muscular substance, of course) between two successive years remain practically uniform in amount, that the corresponding additions to lung capacity show a uniform and steady decline from the beginning

to the end. The ratio between these two items, in other words, as represented by the figures in the two tables, would indicate a steady and continued decrease in the value of the vital index directly due and traceable to the quality of the exercise which was performed.

If, therefore, the significance of this vital index as a criterion of good training is maintained, then our system of physical training would clearly have the fault of adding on too much weight. I would not be willing to admit the other alternative—namely, that the development of the lung capacity was in any way neglected or its importance underestimated. On the contrary, all possible means of training and instruction are employed to develop the lung capacity to the greatest possible extent. Thus, for example, after this observation was made for the first time, more running was practised than had been done the year previous; but still the results remained obstinately the same, and the vital index invariably became smaller in those of the cadets who had gained both weight and strength in amount far above the average. This was noticed and expressed in my first publication on the subject,* and has since been noticed to recur every year with regularity.

I was then, and am now, a firm believer in the value of this index, because it is based upon sound physiological principles and because of the high authority from whom it has emanated.

If the results obtained from our method of training show a decrease in the index, which results are contrary to the expectations, we should be inclined first to look for the fault in our methods of training rather than to question the validity of this index as a test of the condition of training.

It is likely, or at least more than merely possible, that we may here touch one of the more vital points in connection with the whole subject of physical training. If, for instance, we admit, as I think we must, that an increase in muscular strength is necessarily accompanied with a proportionate increase in the weight of our muscles, whether this ratio is a decreasing one or not; if, furthermore, this increase in weight be out of proportion to the simultaneous increase

* See *Surgeon General's Report to the Secretary of the Navy*, 1893, p. 155.

in the lung capacity, and, consequently, result in a decrease in the vital index—it is, as mentioned before, more than merely possible that a person's capacity for carrying both strength and weight has been reached to the fullest extent consistent with the most perfect health of that person.

If by further researches this supposition could be proved as correct, we should be in possession of a most valuable means for determining the weight and strength capacity for every person undergoing physical training. We should then, moreover, be in possession of a simple means by which we might pronounce judgment that is worthy of the name on the comparative merits of the different so-called "systems" of gymnastics in vogue, thus forever putting an end to that everlasting war of words that has been waged about them for very nearly one hundred years without making us any wiser.

TABLE IV.—*Total Strength.**

		MEAN INCREASE IN KILOS BETWEEN THE YEARS					
		16–17.	17–18.	18–19.	19–20.	20–21.	Total.
1	Records of 605 cadets, normal values.....	39	56	43	69	61	268
2	Records of 188 observations in gymnasium.....	266	200	236	230	244	1176

The foregoing Table IV shows plainly that exercise is followed by a very large increase in muscular strength. Advancing years, at least within the limits of those presented by the table, seem to make no difference in the amount of gain in strength, as is the case with the other items treated of in this paper. The average annual increase in total strength that was calculated from six hundred and five cadets for the years from sixteen to twenty-one is 55·6 kilos; that due to gymnasium exercise is 235 kilos, exceeding the normal nearly five times. Indeed, the increase in strength following exercise which is systematized and well regulated is generally so rapid and occurs with such great regularity that it has long since ceased to astonish

* For an explanation of what is meant by "total strength," the reader is referred to an article in the *Amer. Jour. of the Med. Sciences*, September, 1894—Football and the Physique of its Devotees, etc., by H. G. Beyer.

me, and I firmly believe that the now so wonderful performances of most of our "strong men" are well within the reach of the majority of healthy men, if such performances were a serious enough part of their ambition to make them do the exercises necessary to develop them. Nothing, in fact, seems to be more natural to our composition and organization than to immediately provide an increased supply of strength in answer to an increased demand made upon it through the performance of muscular work. This is true not only for the period of normal growth which comes to an end at the age of twenty-one years, but also for that period which reaches well into middle life.

A STATISTICAL AND EXPERIMENTAL STUDY OF TERMINAL INFECTIONS.*

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The recognition of the part played by bacterial infection in the causation of death in chronic diseases has gained in importance with the multiplication of observations upon the occurrence of such infections in human beings, the subjects of chronic diseases of the heart, kidney, liver, and other organs. The data for a proper appreciation of the relation existing between bacterial infection and chronic disease are to be gained less by the clinical observation of cases than by systematic bacteriological examinations at autopsies. The routine study of cases at autopsy by bacteriological methods will not infrequently reveal the presence of pathogenic micro-organisms which were, perhaps, unsuspected during the life of the patient. This fact has been demonstrated repeatedly in the course of the autopsy work at the Johns Hopkins Hospital, where for some time past it has been the practice to make routine bacteriological examinations. Indeed, such examinations have come to be regarded as belonging to the technique of the post-mortem examination. The data which the first part of this paper embodies are taken from the pathological records of the hospital, and represent the results of bacteriological examinations made by Drs. Welch, Councilman, Barker, Blumer, Livingood, and myself.

The routine bacteriological examination of ordinary cases is readily made and does not take much time, while the investigation of general, multiple, and mixed infections requires much time and patience. The proper study of cases demands the preparation of cul-

* Read at the Eleventh Session of the Association of American Physicians held at Washington, April, 1896.

tures from the various cavities and all the viscera of the body, and this is equally true whether a definite local infectious process is discovered or not. The distribution of bacteria is not always a regular one, and with a given local inflammatory process of bacterial origin secondary bacterial accumulations may be present in some organs and absent from others. Where no such special localization occurs only a complete set of cultures can be taken to exclude the possibility of bacterial development.

Negative results in bacteriological examinations do not necessarily afford positive proof of the absence of micro-organisms, as these may have been present in such small numbers as to escape detection, or have been irregularly distributed in an organ, or at the time of examination have been dead; or, finally, the culture media upon which they have been transplanted may not have been suitable to their growth. In view of some of these possibilities, cover-slip preparations from organs and exudates should be made and examined along with the study of cultures.

Out of the 793 autopsies performed in the Johns Hopkins Hospital I have found in the records 255 in which occurred chronic heart or kidney disease, or both combined, and in which the bacteriological examination was sufficiently complete to make them of use for this paper. This number does not represent all the cases of chronic heart and kidney disease that came to autopsy. In some the bacteriological examination was either not carried out at all or the records do not suffice for our purpose. Tubercular infection is not included in this summary, although we are not unmindful of the occurrence of acute miliary tuberculosis as a terminal event in cases with some focus of localized tuberculosis. Of the 255 cases mentioned, 213 gave positive and 42 negative results.

If we direct our attention to the results of the study by bacteriological means of cases of chronic Bright's disease, in which the kidney lesions were the chief feature, we find that of 32 cases unassociated with cardiac or other chronic disease, 29 gave positive and 3 negative results. Similarly of 112 cases of combined chronic renal and chronic cardiac disease, 85 yielded positive and 27 negative re-

sults. In a considerable number of cases of chronic Bright's disease other chronic diseases were present. These consisted of tumours, such as carcinomata, sarcomata, and myomata, of cirrhosis of the liver and lungs, chronic proliferative peritonitis, etc. Of 54 cases of combined chronic Bright's disease, bacteriological examination gave 51 positive and 3 negative results.

A similar study of the cases of chronic heart disease yields the following:

The number of cases of heart disease alone in which bacteriological examinations were made was 41, of which 32 were positive and 9 negative. The number of cases of chronic heart disease associated with other chronic diseases than chronic Bright's disease was 22, of which 16 gave positive and 6 negative results. The cases of combined chronic heart and kidney diseases are the same as are given with the summary relating to the latter organ.

For the purpose of this paper no attempt was made to separate the primary heart from the primary kidney cases where both organs were affected. Again, the cardio-vascular system has been regarded as a whole, no division having been made into primary arterial and primary cardiac disease, provided that the heart was the seat of pathological changes. These latter consisted of hypertrophy with and without dilatation, disease of the coronary arteries, fibrous myocarditis, and chronic adhesive pericarditis.

The infections may be local or general. The former are much more common than the latter, and are found in a large proportion of all cases of chronic Bright's disease, arterio-sclerosis, cirrhosis of the liver, and other chronic diseases. Affections of the serous membranes (acute peritonitis, pleuritis, and pericarditis), meninges, and endocardium are the most frequent, but not the only lesions. Special localizations of the micro-organisms are met with in the viscera.

We shall first consider the general infections. No special group of bacteria is present in these in contradistinction to those present in the local infections. Not infrequently a local lesion, such as erysipelas, acute peritonitis, or acute endocarditis, exists which antedates the general invasion of the body by the infectious agent. It, how-

ever, may and actually does happen that the point of entrance of the micro-organisms can not be made out at the autopsy. Either no lesion exists at the place of entrance or, as is more probable, it is so small and insignificant as to escape detection. Among the cases of general infection are found examples of cryptogenetic infection, in which the primary focus is so hidden that it is found at autopsy only after painstaking search.*

In the series of chronic kidney diseases are included 38 cases of general infection: namely, Bright's disease alone, 3 (out of 29); combined Bright's and heart disease, 19 (out of 85); combined renal and other chronic diseases, 16 (out of 51).

The micro-organisms causing these infections, 38 in all, were the streptococcus pyogenes, 16 cases; staphylococcus pyogenes aureus, 4 cases; micrococcus lanceolatus, 6 cases; gas bacillus, three times alone and twice combined with the bacillus coli communis; the gonococcus, anthrax bacillus, bacillus proteus, the last combined with the bacillus coli, the bacillus coli alone, a peculiar capsulated bacillus, and an unidentified coccus, each in one instance.

What is striking in this list is the preponderance of the usual pyogenic cocci. These may occur without association with abscess formation. In a large proportion of our cases of septicæmia visible focal lesions within the organs were not present at the autopsy. The usual conditions found were acute splenic tumour and more or less severe parenchymatous degeneration of the viscera, with sometimes microscopical necroses of tissue cells as well.

No attempt has been made to separate the streptococci into distinct groups, and in this paper they are all included under the designa-

* Such a case was that of a young man who had a healed tuberculous lesion of the dorsal vertebræ associated with kyphosis. A general streptococcus infection, unsuspected during life, was found at the autopsy. No source of the infection was present on the surface of the body, in the serous cavities, or in the internal organs. Painstaking search revealed a small streptococcus abscess, embedded in the deep muscles of the back, in the region of the healed tuberculous focus. The heart of the individual was hypertrophied and dilated, the hypertrophy affecting chiefly the right side. The cavity of the thorax was small; the lungs the seat of chronic passive congestion. The myocardium was the seat of fatty degeneration.

tion of streptococcus pyogenes, notwithstanding certain cultural differences noted in the behaviour of specimens from different sources. For the most part the streptococci were not highly pathogenic for laboratory animals.

The characters of the staphylococcus aureus are so well marked that no trouble is found with its classification.

The micrococcus lanceolatus was identified from its morphological and cultural properties, as well as its well-known pathogenic effects upon animals.

Concerning the bacillus aerogenes capsulatus it may be said that there is evidence of the distribution of the bacilli within the body during life, although I am reluctant to admit a general invasion of the blood and tissues before death. The maintenance of life for any length of time after this organism has established itself in the blood and produced gas there is probably impossible.

The cases of proteus septicæmia are of their kind unique, and bear upon the growing belief that this group of bacteria may exhibit distinct pathogenic properties for man. The case of anthrax septicæmia is of interest, as it was associated with an acute endocarditis and peritonitis due to the bacillus anthracis.* Of more than ordinary interest is the case of gonococcus septicæmia with localization upon the heart valves.† A second instance of general infection with the gonococcus following gonorrhœa has just been noted by us. This case will be reported in full later by Drs. Thayer and Lazear.

In a few instances bacteria which could not be brought into harmony with any of the described pathogenic species were isolated, at one time from one or two organs, at another from a local inflammation, or, more rarely still, from several organs, or the heart's blood. These unidentified bacteria are reserved for a fuller description hereafter.

The portals of entry of the micro-organisms in these cases of general infection were in some instances made out and in others not. In some cases localized areas of inflammation existed which were taken

* Reported by Blumer and Young. *Johns Hopkins Hospital Bulletin*, 1895, Nos. 54, 55.

† Reported by Thayer and Blumer. *Ibid.*, 1896, No. 61.

to have antedated the general invasion. Not a few of these demand themselves an explanation of the point of entrance of the infectious agent.

Of the 16 instances of streptococcus infection the infection atria into the general circulation are believed to be as follows: Cellulitis, erysipelas, leg ulcer, bedsore, 7; phthisical cavity, 1; laparotomy, 2; tapping abdomen, 1; acute pleurisy, peritonitis, or pericarditis, 3; sloughing angio-sarcoma, 1; necrosis of placental attachment in uterus, 1.

Of the 4 cases of staphylococcus aureus septicæmia there were: Acute pericarditis following diphtheria, 1; operation upon the perinæum and cervix uteri, 2; undetermined (generalized melanotic sarcoma), 1.

Of the 6 cases of micrococcus lanceolatus infection, acute pneumonia claims 4; acute endocarditis and acute pericarditis, and pleuritis, 1 each.

The 5 gas-bacillus cases give aneurism of the aorta opening externally, amputation of arm, each 1 case, and intestine 3 cases.

The 2 proteus cases take their origin, one in a sloughing bedsore and the other from a chronic diphtheritic colitis.

The capsulated bacillus gained entrance from an ascending pyelonephritis; the anthrax bacillus from an anthrax œdema of the face.

The number of local infections is much larger. Of the cases of chronic Bright's disease alone, 26 occur in which bacteria were present in some local situation; of the cases of combined kidney and heart disease 66, and of kidney and other chronic diseases 35.

The micro-organisms found in the local situations are, for the most part, the same as were met with among the general infections. They are not infrequently associated with one another, and occasionally it happens that the different organs contain different bacteria. Indeed, certain bacteria appear with such uniformity within certain organs that these must be regarded as presenting better opportunities for their growth than do others. Examples are afforded by the colon bacillus which is so commonly found in the kidneys and the lungs, and the occurrence of streptococci in congested and œdematous lungs.

The following table exhibits the variety, combinations, and fre-

quency of occurrence of the micro-organisms found in the local infections:

	Times.
Streptococcus alone	14
Streptococcus and b. coli	15
Streptococcus and staphylococcus	2
Streptococcus and b. lactis aërogenes	1
Streptococcus and b. pyocyaneus	1
Streptococcus and unidentified bacilli	2
Staphylococcus aureus	8
Staphylococcus aureus and b. coli	3
Staphylococcus albus and b. coli	1
Streptococcus, staphylococcus aur., and b. coli	4
Streptococcus, staphylococcus aur., and b. lactis aërogenes	1
Micrococcus lanceolatus	12
Micrococcus lanceolatus and b. coli	3
Micrococcus lanceolatus, streptococcus, and staphylococcus	4
Micrococcus lanceolatus and streptococcus	1
Micrococcus lanceolatus and staphylococcus aureus	1
Micrococcus lanceolatus and b. aërogenes capsulatus	2
Bacillus coli communis	20
B. coli and b. diphtheriæ	1
B. coli, b. diphtheriæ and streptococcus	1
B. coli, streptoc., staphyl. alb., and unidentified bac.	1
B. pyocyaneus and b. coli	2
B. pyocyaneus and b. lactis aërogenes	1
B. pyocyaneus, m. lanceolatus, and b. coli	1
B. pyocyaneus and streptococcus	1
B. proteus	1
B. proteus and b. coli	1
B. proteus and b. pyocyaneus	1
B. aërogenes capsulatus	1
B. aërogenes capsulatus, b. coli, and staphylococcus	1
B. aërogenes capsulatus, b. coli, and streptococcus	1
B. influenzæ	2
B. diphtheriæ	1
Staphylococcus cereus flavus	2
B. tetani, streptoc., s. aureus, b. coli	1
Gonococcus, staphyl. alb., and streptococcus	1
Undetermined bacilli	3

Upon analyzing this group we find that at least one place of localization is found in the following situations with the frequency mentioned:

	Cases.
Acute peritonitis	37
Acute pleuritis (without pneumonia)	11
Acute pericarditis	23
Acute endocarditis	19
Acute meningitis	4

In reference to this group, it may be said that along with the micro-organisms in the foci of inflammation the same bacteria as are present there may appear in one or more of the organs of the body. Their distribution is, however, not so general as to warrant their classification among the true septicæmias.

The varieties of bacteria which are present in these situations is shown by the following summary, which also exhibits the portals of entry of the micro-organisms so far as they could be determined with some probability:

ACUTE PERITONITIS.

Bacteria.	Frequency.	Infection atrium.	
Streptococcus	8	Intestine	13 times.
Staphylococcus aureus and albus	9	Laparotomy	13 "
Micrococcus lanceolatus	4	Tapping abdomen	2 "
Bac. aërogenes capsulatus	2	Pneumonia	3 "
Bac. coli communis	3	Sloughing myoma uteri	2 "
Bac. pyocyaneus	1	Pyelonephritis	1 time.
Bac. proteus	1	Doubtful	3 times.
Bac. anthracis	1		
Staphylococcus cereus flavus	1		
Streptococcus and staphyl. aureus	2		
Streptococcus and b. coli	1		
Streptococcus, staphyl. aur., and b. coli	1		
Streptococcus, staphyl. alb., and undetermined bacilli	1		
Bac. pyocyaneus and b. coli	1		
Unidentified bacilli	1		

ACUTE PERICARDITIS.

Bacteria.	Frequency.	Infection atrium.	
Micrococcus lanceolatus	11	Pneumonia	8 times.
Streptococcus	4	Bronchitis	2 "
Staphylococcus aureus	1	Erysipelas	1 time.
Bac. pyocyaneus	1	Leg ulcer	1 "
Bac. influenzæ	1	Tonsils	1 "
M. lanceolatus and b. coli	1	Peritonæum	1 "
Streptococcus, staphyl. aur., and b. coli	1	Cancer stomach	1 "
Staphylococcus and b. coli	2	Sloughing myoma	1 "
Unidentified bacilli	1	Doubtful	7 times.

ACUTE ENDOCARDITIS.

Bacteria.	Frequency.	Infection atrium.	
Micrococcus lanceolatus	5	Pneumonia	4 times.
Streptococcus	7	Intestine	1 time.
Staphylococcus aureus	2	Ulcer leg	1 "
Bac. influenzæ	2	Sloughing carc. uterus	1 "
Streptococcus and staphylococcus	1	Abscess liver	1 "
Bac. pyocyaneus and b. coli	1	Carcinoma pylori	1 "
Undetermined	1	Doubtful	10 times.

ACUTE PLEURISY.

Bacteria.	Frequency.	Infection atrium.	
Streptococcus	4	Peritonæum	4 times.
Micrococcus lanceolatus	2	Intestine	3 "
B. coli com.	1	Bronchitis	1 time.
B. proteus	1	Infarction lung	1 "
Streptococcus, staphyl. aur., b. coli	1	Doubtful	2 times.
B. aërog. caps. and b. coli	1		
B. coli and unidentified bacillus	1		

ACUTE MENINGITIS.

Bacteria.	Frequency.	Infection atrium.	
Micrococcus lanceolatus	3	Ac. lobar pneumonia	3 times.

The intestine, as will appear from these statements, is regarded as the portal of entry not only of many of the bacteria found in the inflamed peritonæum, but also of some of those present in the pleura, upon the heart valves, and within the organs. This conclusion is based in part upon the behaviour of those species which are known to be derived from the intestine, namely, the colon group of bacilli. It has been found that these bacteria wander through the intestinal walls with great regularity where lesions of the intestinal mucosa exist. The lesions present here need not for this purpose be considerable, as localized areas of hyperæmia and small hæmorrhages often suffice to open the way for their escape; in grave lesions, such as necroses, ulcerations, and tumours, the colon bacilli are found almost without exception in some of the distant viscera. It is considered probable that the definitely pathogenic species of bacteria which are not infrequently associated with the colon bacilli in the intestine may find similar, although probably not identical, opportunities for escape.*

The occurrence of the colon bacilli in the organs is frequently unassociated with any lesion referable to their presence. Indeed, in the great majority of cases we have not looked upon the presence of a moderate number of colon bacilli as of pathological significance. In a few cases, however, they were present so generally in the body and in such large numbers that they could not be disregarded, and in rare instances they were found in association with definite lesions in such a manner as to leave no reasonable doubt of their pathogenic character.

* See Flexner, Peritonitis caused by the Invasion of the Micrococcus Lanceolatus from the Intestine. *Johns Hopkins Hospital Bulletin*, 1895, No. 49.

On the other hand, the frequent occurrence of the colon bacillus in combination with the pyogenic cocci is a matter of considerable importance, and, under such circumstances, these micro-organisms may come to have greater significance.

The majority of cases of acute pericarditis and acute endocarditis are found at the autopsy to be associated with pneumonia, either lobar or lobular in nature. As the micro-organisms present in the several lesions are usually the same, it is probable that the pneumonia is the source of the pericardial and endocardial infection. Cases of pleurisy associated with pneumonia have not been included in our list. In some instances acute pericardial and endocardial inflammations have been met with independently of pneumonia, and these may, indeed, be the sources from which other serous surfaces become infected, or a general infection arises.

In cases of heart disease uncomplicated with kidney disease, terminal infections are often encountered. Of 41 cases of arterio-sclerosis and chronic valvular disease, cultures showed in 32 the presence of bacterial infection, while in 22 cases of cardio-vascular disease, associated with some other chronic condition than Bright's disease, positive results were obtained in 16. In these 48 positive cases the distribution of the bacteria was general in 14 instances. The micro-organisms associated with these septicæmias were streptococcus pyogenes, 9 cases; micrococcus lanceolatus, 2 cases; staphylococcus pyogenes aureus, 1 case; staphylococcus albus and streptococcus, 1 case; bacillus coli communis, 1 case.

The portals of entry in the above cases are believed to have been as follows:

Streptococcus	Leg ulcer, ulcer scrotum, circumcision	3
	Laparotomy and peritonitis	1
	Abscess of back (kyphosis)	1
	Tonsil	1
	Carcinoma of stomach	1
	Sloughing carcinoma of neck	1
	Otitis media	1
	Doubtful	1
Staphylococcus aureus	Fracture of skull and acute meningitis.	
Micrococcus lanceolatus	Doubtful (no pneumonia).	
Bacillus coli communis	Intestine.	

The remaining 32 cases of infection were of local nature, and of this number 22 occurred in uncomplicated cardio-vascular disease, and 10 in combined chronic cardiac and other disease. As in the case of the summary of renal diseases, we find here also that the variety of infecting bacteria is greater than in the cases of general infection. The following gives the kind of bacteria and the frequency and manner of their occurrence:

Bacillus coli communis	4
Streptococcus pyogenes	4
Micrococcus lanceolatus	4
Bacillus lactis aërogenes	1
Bacillus pyocyaneus	1
Staphylococcus pyogenes aureus	2
Staphylococcus pyogenes aureus and bacillus coli	1
Staphylococcus pyogenes aureus, streptococcus, gas bacillus, and bacillus coli	1
Streptococcus and gas bacillus	1
Streptococcus and bac. fœtidus	1
Bacillus coli and liquefying bacillus (undetermined)	1
Undetermined bacilli	3

I would direct attention to the fact which must now be admitted, that the bacillus pyocyaneus which occurs several times in our tables is like the more common pyogenic organisms, capable of producing distinct and widespread lesions in human beings. We have met with several instances in which extensive necrosis, associated at times with ulceration, had been caused by it in the gastro-intestinal tract.

The special places of localization of the bacteria in the last series of cases is shown by the following summary:

Acute peritonitis	9
Acute pleuritis (without pneumonia)	6
Acute pericarditis	8
Acute endocarditis	15
Acute meningitis	3

The micro-organisms which were isolated from each of the above situations were the following:

Acute Peritonitis.

Bacteria.	Frequency.	Infection atrium.	
Streptococcus	5	Gastric ulcer	1 time.
Bacillus coli communis	3	Laparotomy	5 times.
Streptococcus and bac. fœtidus	1	Carcinoma stomach	1 time.
		Circumcision	1 "
		Doubtful	1 "

ACUTE PERICARDITIS.

Bacteria.	Frequency.	Infection atrium.	
Micrococcus lanceolatus	5	Pneumonia	2 times.
Streptococcus	1	Tonsil	1 time.
Bacillus coli	1	Bronchitis	1 "
Gonococcus	1	Peritonæum	1 "
		Urethra	1 "
		Doubtful	2 times.

ACUTE ENDOCARDITIS.

Bacteria.	Frequency.	Infection atrium.	
Micrococcus lanceolatus	7	Pneumonia	3 times.
Streptococcus	5	Pleurisy	1 time.
Gonococcus	1	Bronchitis	1 "
Streptococcus, staphylococcus aureus, and b. influenza	1	Tonsil	1 "
		Otitis media	1 "
Chain bacilli (unidentified)	1	Circumcision	1 "
		Urethra	1 "
		Acute endometritis	1 "
		Carcinoma uteri	1 "
		Doubtful	4 times

ACUTE PLEURISY.

Bacteria.	Frequency.	Infection atrium.	
Streptococcus	3	Bronchitis	1 time.
Micrococcus lanceolatus	2	Circumcision	1 "
Gonococcus	1	Urethra	1 "
		Doubtful	3 times.

ACUTE MENINGITIS.

Bacteria.	Frequency.	Infection atrium.	
Micrococcus lanceolatus	2	Pneumonia	1 time.
Streptococcus	1	Fracture of skull	1 "
		Operation on pharynx	1 "

This list leaves unmentioned a considerable number of cases in which, in the absence of micro-organisms in or inflammation of the serous cavities and upon the heart valves, pathogenic bacteria were found in one or several of the viscera. It would lead too far afield to consider the character of the lesions which may be associated with the invasion of the pathogenic bacteria which we have considered. But beside those which were at once evident to the naked eye, microscopic foci of cell degeneration and necrosis were frequently encountered.

The summary presented above will, if I mistake not, suffice to show the value of systematic bacteriological studies at human autopsies. It will also serve to emphasize the importance of the rôle played by pre-existing chronic disease in determining susceptibility to infec-

tion, and to illustrate the statement of Osler: "It may seem paradoxical, but there is truth in the statement, that persons rarely die of the disease with which they suffer. Secondary infection, or, as we are apt to call them in hospital wards, terminal infections, carry off many of the incurable cases in the wards." *

Now that the subject of the intoxications is coming to have so much significance in human pathology, and there is reason to believe that many of the bacterial infections do harm by intoxication rather than by infection, *per se*, it might be pertinent to ask how far the operation of toxic substances may be responsible for still other examples of death in chronic disease, and whether there exists under these circumstances greater vulnerability to the actions of these poisons just as we must admit a lessened resistance to the invasion of pathogenic bacteria.†

The exact factors concerned in producing the increased susceptibility to infection in chronic disease are quite unknown. That the lessened resistance depends upon profound alterations in the body constituents is certainly probable, and, in view of the disturbance of function associated with changes in organic structure, it is not difficult to form conjectures as to their possible character. We are also helped to a conclusion by the experiments upon animals, which tend to show that various injurious influences, by modifying the quantity and quality of the blood, the physiological nutrition and state of rest of the body, as well as its normal metabolism, increase susceptibility to infection.

It is exceedingly probable that pathogenic micro-organisms not infrequently gain entrance within the body in health, but do little harm there, and are quickly removed or destroyed. We have abun-

* Osler, Practice of Medicine, 1895, p. 132.

† Cf. Bouchard, Leçons sur les auto-intoxications dans les maladies. Paris, 1887. Roger, Action du foie sur les poisons. Paris, Thèse, 1887. Also Rôle du foie dans les auto-intoxications. *Gaz. des hôpitaux*, 1887, p. 66. Ewald u. Jacobson, Ueber ptomainartige Körper im Harn bei chronischen Krankheitsprocessen. *Berliner klin. Wochenschrift*, 1894, No. 2, p. 25. Albu, Ueber die Auto-intoxicationen der Intestinaltractus. Berlin, 1895. Kraus u. Honigmann, Pathologie der Auto-intoxicationen. *Ergebnisse der Allg. Pathologie u. Pathologischen Anatomie*, Zweite Abtheilung, 1895, p. 573.

dant evidence of the power of the body to destroy many pathogenic bacteria which enter it from without or are introduced by experiment, and thus the conclusion that in chronic disease the mechanisms of disposal of bacteria are in abeyance or insufficient is not to be resisted.

The chief defences against the invasion of pathogenic micro-organisms are the body fluids and cells. To this activity of the juices of the body and certain of its constituent cells is doubtless owing our relative immunity from living disease germs. It is therefore of the first importance to know whether under the conditions which have been shown to predispose to infection there is a demonstrable difference in the bactericidal power possessed by these substances.

The experiments which will be given here have been made with the blood-serum of a number of cases of chronic disease from the medical wards of the Johns Hopkins Hospital. I wish to express my indebtedness to Dr. Osler for his courtesy in placing these patients at my disposal, and to the house staff for their ready co-operation. For the purpose of comparison, blood obtained in surgical operations from healthy individuals was employed. For the placental blood I am indebted to Dr. Dobbin, the resident obstetrician, who collected it for me in sterile tubes immediately after cutting the umbilical cord.

The blood of adults was obtained directly from veins by aspiration.* A Roux syringe, sterilized by steam, was employed, the skin of the patient first having been scrupulously cleansed. This method is believed to have advantage over cupping as employed by Stern. Contaminations of the blood were never encountered, and 10 cubic centimetres, or even more, were readily secured at a single operation. The blood was immediately transferred to Nuttall's bulbs, as it was found that the serum separated better in these than in test tubes. After a period varying from two to thirty hours the serum was pipetted off into test tubes, 1 cubic centimetre being the standard amount used in each experiment.

* In two instances the blood was aspirated immediately after death from the heart, and in another instance from the femoral vein. In all cases only sterile blood was employed, cultures upon agar-agar being employed for this determination.

The bacteria which I have thus far tested upon human blood-serum are: (1) staphylococcus pyogenes aureus, (2) bacillus typhosus, (3) bacillus of Friedländer. The experiments are most complete as regards the first-mentioned micro-organism, and the present report will refer exclusively to it. The reasons which led to the selection of the staphylococcus aureus for the first series of experiments are the following: The study of the cases of terminal infections in human beings showed that the pyogenic cocci were the chief bacteria concerned with such infections. It was therefore considered most natural to expose these to the action of the serum. The staphylococcus aureus is not the chief invader in these infections; it is exceeded both by the streptococcus pyogenes and the micrococcus lanceolatus. However, the development upon culture media of the last two forms is subject to such variation under favourable conditions that the error of experiment must have been much greater than with the golden staphylococcus, which presents no such difficulties.

Writers do not entirely agree as to the effects of the normal fluids of the body upon the pyogenic cocci, the majority (Nuttall,* Stern,† Prudden ‡) stating that they are quite without effect. On the other hand, Rovighi # considers that he has shown human blood-serum to possess bactericidal properties for the staphylococcus aureus. He also observed a reduction of this power for the staphylococcus aureus in two out of six persons suffering from severe cachexia.

In our experiments the blood of a number of persons has been subjected to study, and from the results obtained it will appear that normal human blood-serum does possess distinct bactericidal properties for staphylococcus aureus; and also that this power is absent, or diminished, in at least some cases of advanced chronic disease.

Our experiments show that the destructive effect exerted by the normal blood-serum upon the staphylococcus aureus is not an invariable quantity, and is at most much less marked than is observed with

* *Zeitschrift für Hygiene*, 1885, Band iv, p. 353.

† *Zeitschrift für klin. Medicin.*, 1890, Band xviii, p. 46.

‡ *Medical Record*, 1890, vol. xxxvii, p. 85.

\# *Atti della R. Accademia medica di Roma*, Anno 16, vol. 5, Serie 11, Rom., 1890.

some other pathogenic bacteria, such as the bacteria of Asiatic cholera and of typhoid fever. It is, however, unjustifiable to compare, as has been done, the reduction in numbers which takes place when the golden staphylococcus is transplanted to blood-serum, with the diminution which regularly occurs after the introduction of the micro-organism into physiological salt solution. The two processes have little in common; the one fluid (blood-serum) is at first destructive, and, after spending its power in this direction, becomes a suitable culture medium, while the other (salt solution) permits at first a small increase of organisms from the nutriment carried over, and then leads in a relatively short time to a gradual, but often complete, disappearance from starvation of the added micro-organisms.

The method of determining the effect of the human serum was the one usually employed. It consisted in introducing a suspension of a young agar or potato culture of the staphylococcus aureus in physiological salt solution into the blood-serum. The same oese was used throughout each series of experiments, and care was exercised not to carry any of the nutritive material into the inoculated serum. Duplicate sets of plates were commonly employed. The inoculated blood-serum was kept at the temperature of 35.5° to 36° C. The agar-agar plates were permitted to develop for at least forty-eight hours at this temperature. For the enumeration of crowded plates the counter of Lafar * was used under a very low power (Ziess objective A_2, eye-piece No. 2) of the microscope. I also found this instrument very convenient for counting with the naked eye when a moderately large number of colonies had developed upon the plates. This method of estimation is not free from error; but when carefully conducted the duplicate counts are in remarkable accordance with each other.

Action of Normal Human Serum upon Staphylococcus Aureus.

No.	Control plate.	1 hour.	2 hours.	4 hours.	6 hours.	24 hours.
1	33,764	.....	.....	4,823	11,255	Increase.
2	25,272	.....	18,925	8,424	4,212	Increase.
3 †	10,920	7,800	7,210	5,740	.....	Increase.

* *Zeitschrift für Nahrungsmittel-untersuchung.*, Wien., 1893, No. 24, p. 429. (Abst. *Centralbl. f. Bakteriologie u. Parasitenkunde*, 1894, Band xv, p. 331.)

† Blood from the enlarged veins in a myoma of the uterus.

Action of Placental Blood-serum upon Staphylococcus Aureus.

No.	Control plate.	2 hours.	3 hours.	4 hours.	6 hours.	24 hours.
1	33,260	34,590	.....	39,912	.....	Increase.
2	73,632	67,494	.....	77,511	156,000	Increase.
3	46,566	.....	.....	56,000	200,000	Increase.
4	210,432	.....	.....	280,000	.....	Increase.

Action of Human Serum from Cases of Chronic Diseases upon Staphylococcus Aureus.

No.	Control plate.	1 to 2 hours.	3 to 4 hours.	6 hours.	8 hours.	24 hours.
1*	33,764	.....	28,137	.....	4,220,500	Increase.
2†	57,720	52,000	.....	Increase.	.....	Increase.
3‡	20,614	17,179	30,000	75,000	.....	Increase.
4#	11,154	13,013	.....	.....	50,193	Increase.
5‖	50,000	.....	63,000	.....	.....	Increase.
6△	62,400	51,434	.....	100,000	.....	Increase.
7◊	26,676	15,246	.....	10,166	6,356	Increase.
8‡	66,456	32,000	.....	16,000	.....	Increase.
9↕	14,742	9,828	952	.....	.....	Increase.

* Arterio-sclerosis; mitral insufficiency; failure of compensation.
† Arterio-sclerosis; chronic nephritis.
‡ Arterio-sclerosis; multiple aneurism formation; chronic nephritis.
# Aortic insufficiency.
‖ Chronic nephritis; uræmia.
△ Chronic nephritis; general anasarca.
◊ Arterio-sclerosis; aortic insufficiency. (Discharged improved.)
‡ Emphysema; heart hypertrophy. (Discharged improved after bloodletting.)
↕ Aortic and mitral insufficiency.

The foregoing tables show that of three specimens of average normal human blood-serum one only did not possess marked destructive power for the staphylococcus aureus, and with respect to this sample it may be questioned whether it is to be regarded as strictly belonging to this class.* The greatest number of micro-organisms destroyed was 29,000 in four hours (Case I), and 21,000 in six hours (Case II). Of nine samples of blood taken from persons suffering with some form of chronic disease, in six no appreciable effect was exerted upon this micro-organism. Regarding the three samples in which more or less bactericidal effect was produced, it is of interest to record that in two (No. 7 and 8) the patients improved while in the hospital, and in one instance only (No. 9), in which the blood-serum exhibited distinct bac-

* The figures given in the first table may be taken as representative, as several subsequent estimations confirm them. In one case only (convalescent from measles) was this power missed in normal adult human serum.

tericidal effects, did the patient die within a short time of the examination. It is not a little surprising, in view of the other results given, to find that placental blood-serum exerts at most a temporary inhibition of development of this organism. On the other hand, the placental serum showed marked destructive power for the bacillus typhosus.

Time does not permit a detailed consideration of the relation of immunity to the bactericidal property of the blood-serum, and, indeed, it is admitted that there is no fixed relation between these phenomena. Yet observations are not wanting to show that in both natural and acquired immunity a correspondence between this property of the blood-serum or its absence exists; however, not in all cases. The serum of the rat is destructive to anthrax bacilli, while that of man and the mouse have little or no effect upon these micro-organisms, and the serum of the rabbit varies in its behaviour. Similarly the blood-serum of the immune guinea-pig, when diluted with bouillon, exerts injurious effects upon the cholera vibrio, which the normal serum fails to do.* The serum, too, of the vaccinated calf destroys the activity of the vaccine virus, while the ordinary serum leaves it unaffected.† There is much significance, too, in the observation that just those micro-organisms which are readily and quickly killed by the serum are rarely or never found in the circulating blood in their respective diseases. Examples of this are furnished by Asiatic cholera and typhoid fever. Further, Flügge has pointed out that in anthrax infection in rabbits the bactericidal effect of the blood is lost before the bacilli make their way into the larger vessels.

Normal blood-serum contains, in addition to bactericidal principles, antitoxic substances which must be of great importance in establishing and preserving immunity.‡ The comparative study of the antitoxic values of blood derived from persons suffering from chronic disease is reserved for a later communication.

* Pfeiffer, *Deutsche med. Wochenschrift*, February 13, 1896.

† Sternberg, Immunity and Serum Therapy, New York, 1895, p. 234.

‡ Escherich u. Klemensiewicz, *Centralbl. für Bakteriologie*, 1893, Band 13, p. 153. Abel, *Deutsche med. Wochenschrift*,, 1894, Nos. 48 u. 50, p. 899 u. 936. Wassermann, *Zeitschrift für Hygiene*, 1895, Band 19, p. 408. Fischl u. v. Wunschheim, *Zeitschrift für Heilkunde*, 1895, Band 16, p. 429.